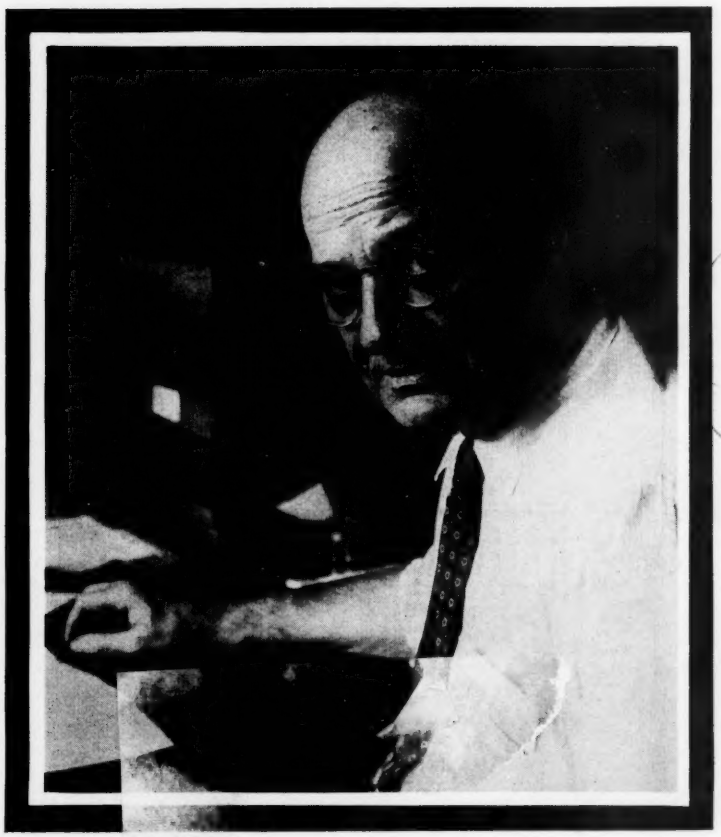
DIABETES

The Journal of the American Diabetes Association



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Plasma Insulin Activity after Glucose

An Index of Insulogenic Reserve in Normal and Diabetic Man

Holbrooke S. Seltzer, M.D., and Walter L. Smith, M.S., Dallas

Time will finally tell whether the net deficiency of insulin activity characterizing human diabetes mellitus is fundamentally due to decreased secretion of the hormone by failing beta cells, or to increased peripheral destruction of insulin produced in normal amounts. Until then, however, preponderant evidence indicates that the functional abnormality is deterioration of a normal quantitative relationship between the blood glucose concentration and the magnitude of insulin secretion.

Anderson[1] and Brown and his associates[2] showed that pancreatic perfusion with glucose caused acute and sustained elevation of insulin secretion, as evidenced by peripheral hypoglycemia in test animals. In confirmation, Metz[3] recently found rapid and prolonged rise of insulin activity in pancreatic venous blood during peripheral infusion of glucose into dogs. Continuous hyperglycemia, however, has experimentally induced degranulation of beta cells,[4] "hydropic degeneration" (glycogen infiltration) of the islets,[2,5] and complete exhaustion of the betacytotropic mechanism resulting in permanent diabetes.[5] The clinical counterparts of these observations were elucidated independently by Bornstein[6,7] and Vallance-Owen.[8,9] These investigators share major credit for establishing that fasting blood of both normal men and elderly diabetics contains significant insulin activity, which increases after glucose feeding, whereas juvenile diabetics have no circulating insulin activity either before or after a glucose load.

The present study was designed to correlate the insulin-secreting capacity of normal subjects and diabetic patients with clinical criteria of carbohydrate tolerance. It was found that diabetics of increasing severity revealed diminishing insulogenic reserve not only by progressively lower titers of fasting plasma insulin activity, but also by proportionately smaller increments of circulating insulin activity in response to a glycemic stimulus.

Presented at the Nineteenth Annual Meeting of the American Diabetes Association in Atlantic City on June 6, 1959.

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MATERIALS AND METHODS

Subjects and patients were all males, who were classified into four groups (table 1). Twelve metabolically normal subjects served as controls. Nine elderly diabetic patients had moderately elevated fasting blood sugars which subsequently were easily controlled by diet alone, or diet and daily tolbutamide (tolbutamide responders). Six other elderly diabetics had higher fasting blood sugars which showed only minimal or transient depression on later combined diet and tolbutamide therapy (tolbutamide nonresponders). Five juvenile diabetics promptly developed severe ketonuria when exogenous insulin was reduced. The mean age of respective groups was 41, 54, 52 and 38 years. Only two patients were obese. No elderly patient received insulin within a week before the test. One patient with juvenile diabetes (MER) tolerated complete withdrawal of insulin, but the others required periodic small injections of regular insulin daily to prevent development of frank ketoacidosis. No insulin was given later than noon of the day before the test. All individuals consumed 300 gm. of carbohydrate and maintenance calories for three days prior to testing.

After an overnight fast, 5 ml. of oxalated blood was drawn for blood glucose, and 50 ml. of heparinized blood for estimation of plasma insulin activity. The patient then swallowed a solution containing 100 gm. of glucose, and repeat specimens were obtained one hour later. Blood glucose was determined in duplicate by the Somogyi-Nelson method.[10] Immediately after obtaining heparinized blood, plasma was separated by centrifugation and stored at 7° C. Specimens were assayed within a week of procurement, and paired fasting and one-hour samples were always run simultaneously. Plasma insulin activity was assayed by determining the glucose uptake from 2 ml. of undiluted plasma by isolated rat hemidiaphragm, adhering strictly to the technic described by Vallance-Owen.[8] Male Wistar rats, weighing between 100 and 130 gm., were used after food was withheld for eighteen to twenty-four hours. Glucose concentration of plasma or buffered salt solution (GBSS)[11] was 300 mg. per 100 ml. Samples of plasma, GBSS alone, or GBSS containing 10, 100, or 1,000

TABLE 1

Increment of blood glucose concentration in normal subjects and diabetic patients one hour after swallowing 100 gm. of glucose.

Patient	Age	Weight (lb.)	Blood glucose (Somogyi—mg. per cent) Fasting	One hour after glucose	Increment (Δ-Gluc.)
		NORMAL SUBJECTS			
DUN	25	145	71	95	24
ENI	37	114	70	145	75
SEL	40	165	91	159	68
PAR	61	128	77	146	69
BEL	43	161	88	128	40
HEN*	39	183	88	163	75
EDM	27	156	76	118	42
SLE	52	144	85	109	24
WAT	34	135	66	120	54
EWI	64	162	80	169	89
DOC	40	168	71	115	44
MOO	32	167	73	118	45
Mean	41	152	78	132	54
±S.E.M.†	± 4	± 6	± 2	± 7	± 6
		ADULT-DIABETIC-TOLBUTAMIDE-RESPONDERS			
SHA	58	140	169	321	152
MUE	54	172	128	241	113
SHE	47	142	195	352	157
GIL	67	147	104	266	162
THO	67	163	172	307	135
SMI	64	162	119	252	133
JOH	46	159	181	386	205
UTZ*	41	253	101	237	136
COO	46	122	120	231	111
Mean	54	162	143	288	145
±S.E.M.	± 3	± 12	± 12	± 19	± 10
		ADULT-DIABETIC-TOLBUTAMIDE-NONRESPONDERS			
SHA	65	156	320	460	140
BRO	59	150	187	267	80
NEI	43	155	288	441	153
RUT	35	167	276	392	116
RIC	67	162	217	335	118
GRE	40	149	243	350	107
Mean	52	157	255	374	119
±S.E.M.	± 6	± 3	± 20	± 29	± 10
		JUVENILE DIABETICS			
MAJ	45	146	298	481	183
MAN	38	157	388	592	204
SCO	25	159	367	452	85
MER	39	156	430	639	209
JAC	42	137	474	607	133
Mean	38	151	391	554	163
±S.E.M.	± 3	± 4	± 30	± 37	± 24

* Patient was obese. † Standard error of the mean.

micro-units of insulin* per ml. of GBSS, were incubated in a Warburg apparatus for ninety minutes at 37.2° C. under an atmosphere of 95 per cent O_2 and 5 per cent CO_2. Residual glucose was determined by the iodometric method of King.[12] Diaphragms were dried for two hours at 110° C., cooled and weighed. Glucose uptake was expressed as micrograms of glucose per 10 mg. of dried diaphragm. "Net glucose uptake" signified total glucose uptake from a sample minus baseline glucose uptake by diaphragm incubated in GBSS alone. Glucose uptake values were converted into micro-units of "effective insulin concentration"† per ml. of plasma

*Five times recrystallized zinc insulin was kindly supplied by Dr. W. R. Kirtley, Eli Lilly and Company, Indianapolis, Indiana.

†(See column 2.) Vallance-Owen[6,13] has emphasized that this method does not measure total insulin content of plasma, but estimates ". . . plasma-insulin activity, or the effective plasma-insulin concentration, i.e., the sum of insulin and its synergists, if any, on the one hand and its antagonists on the other."[13]

from the dose-response curve shown in figure 1. A few glucose uptakes exceeded the equivalent for an insulin concentration of 1,000 micro-units per ml. of GBSS (table 2), but were included as the latter value.

TABLE 2

Plasma insulin activity* before and after ingestion of 100 gm. of glucose

	(A) Mean net glucose uptake (micrograms/10 mg. diaphragm)			(B) Mean effective plasma insulin concentration (micro-units/ml. plasma)		
Patient	Fasting	One hour after glucose	Increment (Δ-G.U.)	Fasting	One hour after glucose	Increment (Δ-P.I.)
NORMAL SUBJECTS						
DUN	189	252	63	240	980	740
ENI	153	184	31	107	215	108
SEL	145	279	134	89	>1,000†	911
PAR	130	250	120	62	940	878
BEL	130	240	110	62	750	688
HEN	123	299	176	53	>1,000	947
EDM	96	359	263	27	>1,000	973
SLE	78	147	69	18	93	75
WAT	74	157	83	16	115	99
EWI	70	187	117	15	230	215
DOC	63	136	73	12	71	59
MOO	56	220	164	11	480	469
Mean	109	226	117	59	573	514
±S.E.M.	± 12	± 19	± 18	± 19	±118	±110
"p"			<0.001			<0.001
ADULT-DIABETIC-TOLBUTAMIDE-RESPONDERS						
SHA	202	257	55	320	1,000	680
MUE	189	245	56	240	830	590
SHE	168	335	167	150	>1,000	850
GIL	158	228	70	120	570	450
THO	115	208	93	43	365	322
SMI	76	119	43	17	48	31
JOH	63	161	98	12	130	118
UTZ	61	150	89	12	100	88
COO	59	107	48	11	36	25
Mean	121	201	80	103	453	350
±S.E.M.	± 18	± 25	± 13	± 38	±136	±103
"p"			<0.02			<0.025
ADULT-DIABETIC-TOLBUTAMIDE-NONRESPONDERS						
SHA	160	205	45	125	340	215
BRO	112	123	11	40	53	13
NEI	76	97	21	17	28	11
RUT	72	87	15	15	22	7
RIC	51	48	— 3	<10‡	<10	0
GRE	37	43	6	<10	<10	0
Mean	85	101	16	36	77	41
±S.E.M.	± 18	± 24	± 7	± 18	±53	± 35
"p"			<0.7			<0.5
JUVENILE DIABETICS						
MAJ	23	71	48§	<10	15	<10
MAN	— 2	—15	0	0	0	0
SCO	—33	4	4	0	0	0
MER	—44	—61	0	0	0	0
JAC	—82	0	0	0	0	0
Mean	—28	0	10	1	3	2
±S.E.M.	± 18	± 21	± 9	± 2	± 3	± 2
"p"			<0.7			<0.6

* Insulin activity expressed both as (A) directly measured net glucose uptake, and as (B) conversion value for effective insulin concentration derived from dose-response curve in figure 1.

† Maximal conversion value was limited to "1,000 micro-units insulin per ml." when net glucose uptake from plasma exceeded glucose uptake for this concentration of insulin in GBSS (figure 2).

‡ Insulin equivalents between 0 and 10 micro-units per ml. were calculated as the latter figure.

§ Only positive increments (values above zero) were used in calculating the average for this group.

RESULTS

Dose-Response Curve (*figure 1*): On a semilogarithmic plot, an almost straight line related mean glucose uptakes for insulin concentrations spanning the physiological range of plasma insulin activity. Respective net glucose uptakes (mean ± S.E.M.) for insulin concentrations of 10, 100, and 1,000 micro-units per ml. of GBSS were 54 ± 14 micrograms per 10 mg. diaphragm; 150 ± 17 micrograms per 10 mg. diaphragm; and 253 ± 22 micrograms per 10 mg. diaphragm.

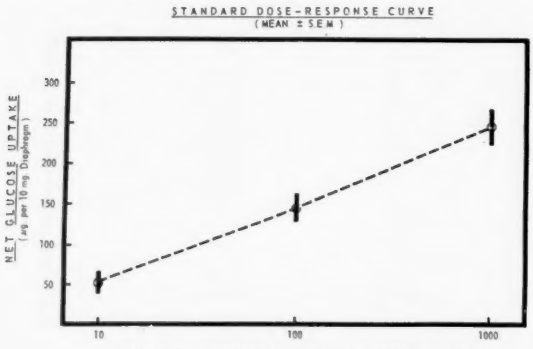

FIG. 1. Virtually a straight line relates net glucose uptakes from logarithmic dilutions of insulin in glucose-buffered-salt-solution (GBSS). Net uptakes from plasma were converted into "micro-units of effective insulin concentration per ml. of plasma" from this dose-response curve.

Fasting Blood Sugar and Increment One Hour After Oral Glucose (*table 1*). Decreased carbohydrate tolerance in successive groups was first manifested by stepwise elevation of fasting blood sugar levels. In normal subjects fasting glucose was 78 ± 2 mg. per 100 ml. (mean ± S.E.M.); in adult-diabetic-tolbutamide-responders it was 143 ± 12 mg. per 100 ml.; in adult-diabetic-tolbutamide-nonresponders it was 255 ± 20 mg. per 100 ml.; and in juvenile diabetics it was 391 ± 30 mg. per 100 ml.

One hour after the oral glucose load, the absolute increase in blood sugar concentration was two or three times higher in diabetic patients than in the control group. The mean "△-blood-glucose" (one-hour value minus fasting value) in normal subjects was 54 ± 6 mg. per 100 ml.; in adult-tolbutamide-responders it was 145 ± 10 mg. per 100 ml.; in adult-tolbutamide-nonresponders it was 119 ± 10 mg. per 100 ml.; and in juvenile diabetics it was 163 ± 24 mg. per 100 ml.

Plasma Insulin Activity Before and After Glucose, Expressed as Net Glucose Uptake (*table 2 and figure 2*). Control subjects and both groups of adult diabetics exhibited insulin activity in fasting plasma, while juvenile diabetics showed none. The mean fasting value for mild adult diabetics was actually slightly higher than the normal mean, and the corresponding level in more severe adult diabetics was below normal, but neither variation was statistically significant. Enhancement of insulin activity after glucose, however, was progressively subnormal in all three diabetic groups. In normal subjects fasting plasma insulin activity (mean ± S.E.M.) was 109 ± 12 micrograms of net glucose uptake per 10 mg. of diaphragm, and one hour after glucose it was 226 ± 19 micrograms per 10 mg. ($p<0.001$). In tolbutamide-responders the fasting level of 121 ± 18 micrograms per 10 mg. had increased an hour later to 201 ± 25 micrograms per 10 mg. ($p<0.02$). Tolbutamide-nonresponders had a fasting value of 85 ± 18 micrograms per 10 mg., which increased minimally to 101 ± 24 micrograms per 10 mg. ($p<0.7$) one hour after glucose. Glucose uptake from fasting plasma of juvenile diabetics was less than from GBSS alone, resulting in a negative value of —28 ± 18 micrograms per 10 mg.; one hour after glucose it was 0 ± 21 micrograms per 10 mg. The mean "△-glucose-uptake" (one-hour value minus fasting value) for respective groups was: normals, 117 ± 18 micrograms glucose per 10 mg. diaphragm; tolbutamide-responders, 80 ± 13 micrograms per 10 mg.; tolbutamide-nonresponders, 16 ± 7 micrograms per 10 mg.; and juveniles, 10 ± 9 micrograms per 10 mg.

PLASMA INSULIN ACTIVITY AFTER GLUCOSE

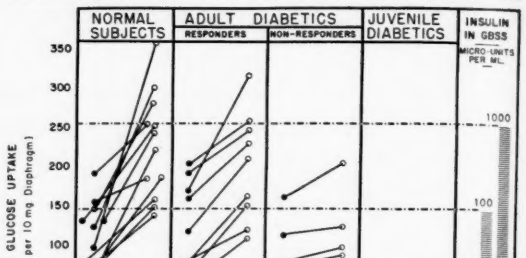

FIG. 2. Solid circles denote fasting plasma insulin activity; open circles are corresponding levels one hour after glucose. Values plotted as net glucose uptakes from plasma. Dose-response curve shown by columns at right is correlated via dashed horizontal lines with glucose uptakes from plasma. At bottom, mean values are in micro-units effective insulin content per ml. plasma, derived from dose-response curve.

Plasma Insulin Activity Before and After Glucose, Expressed as Effective Insulin Concentration (*table 2 and figure 2*). Intra-group and inter-group relationships were similar to those when insulin activity was measured as net glucose uptake, despite limitation of maximal conversion values to 1,000 micro-units per ml. of plasma for net glucose uptakes which were actually higher. In normal subjects plasma insulin activity (mean ± S.E.M., micro-units of effective insulin concentration per ml. of plasma) increased tenfold after glucose, from 59 ± 19 micro-units per ml. fasting to 573 ± 118 micro-units per ml. one hour later ($p<0.001$). Tolbutamide-responders showed a fourfold rise, from 103 ± 38 micro-units per ml. fasting to 453 ± 136 micro-units per ml. at one hour ($p<0.025$). Respective values in tolbutamide-nonresponders were 36 ± 18 micro-units per ml. fasting, and 77 ± 53 micro-units per ml. after glucose ($p<0.5$); and in juvenile diabetics they were 1 ± 2 micro-units per ml. fasting, and 3 ± 3 micro-units per ml. after glucose. The mean "△-plasma-insulin" (one-hour value minus fasting value) was 514 ± 110 micro-units of insulin per ml. of plasma in normal subjects; 350 ± 103 micro-units per ml. in tolbutamide-responders; 41 ± 35 micro-units per ml. in tolbutamide-nonresponders; and 2 ± 2 micro-units per ml. in juveniles.

Index of Insulogenic Reserve (*table 3 and figure 3*). The "insulogenic index" related the amount of increased plasma insulin activity after glucose to the magnitude of the insulogenic stimulus itself. It was calculated by dividing the post-glucose increment of insulin activity (△-glucose-uptake or △-plasma-insulin) by the increment of blood glucose concentration (△-blood-glucose). Figure 3 illustrates that insulin-secreting reserve fell sharply across the spectrum of carbohydrate tolerance, especially when clinical status changed from normal to mildly diabetic. With insulin activity expressed as the more accurate net glucose uptake per 10 mg. diaphragm, the insulogenic index was 2.17 for normal subjects, 0.55 for adult-tolbutamide-responders, 0.13 for adult-tolbutamide-nonresponders, and 0.05 for juvenile diabetics. Relative values were almost identical when increased insulin activity was recorded in more meaningful terms as micro-units of effective insulin content per ml. of plasma, respective indexes being 9.52 for normal subjects (100 per cent); 2.41 for tolbutamide-responders (25 per cent); 0.34 for tolbutamide-nonresponders (4 per cent); and 0.01 for juvenile patients (0.1 per cent).

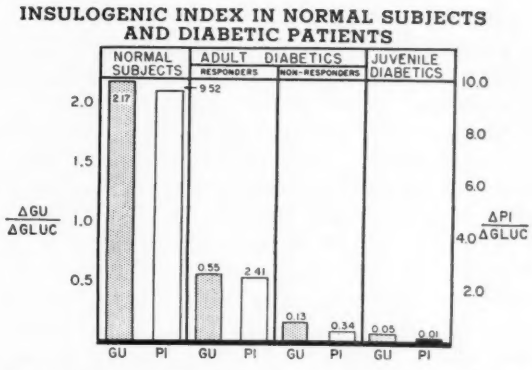

FIG. 3. Dotted columns (GU) show insulogenic index when increment of plasma insulin activity after glucose is in micrograms net glucose uptake per 10 mg. diaphragm (△GU). Open columns (PI) depict index when enhanced insulin activity is expressed as micro-units insulin per ml. plasma (△PI). Blood sugar increment after oral glucose is "△GLUC."

TABLE 3

Index of insulogenic reserve*

Clinical status	Increment† of plasma insulin activity: Glucose uptake (Δ GU) micrograms per 10 mg. diaphragm	Increment† of plasma insulin activity: Plasma insulin concentration (Δ PI) micro-units per ml. plasma	Increment† of blood glucose (Δ Gluc.) mg. per 100 ml.	Insulogenic indexes: Δ GU / Δ Gluc.	Insulogenic indexes: Δ PI / Δ Gluc.	Per cent of normal
Normal subjects	117‡	514‡	54‡	2.17	9.52	100
Adult diabetics						
Tolbutamide-responders	80	350	145	0.55	2.41	25
Tolbutamide-non-responders	16	41	119	0.13	0.34	4
Juvenile diabetics	10	2	163	0.05	0.01	0.1

* Increment of plasma insulin activity per mg. per cent rise of blood sugar after oral glucose load.
† Increment = one-hour value minus fasting value.
‡ Mean values.

DISCUSSION

The present data do not help localize the initial metabolic block which upsets normal pegging of insulin secretory rate to blood glucose concentration. However, the sequence of events in these patients is better explained by assuming primary disruption of beta-cell function than by invoking peripheral insulin antagonists, among which insulinase[14] comes foremost to mind.

Subsequent to Metz' demonstration that normal islets respond rapidly and quantitatively to a rising blood sugar,[8] the authors have also found a tenfold rise in pancreatic vein insulin activity within eight minutes after starting to infuse 50 per cent glucose at 3 ml. per minute into a femoral vein.[15] Rapid release of adequate insulin is clearly an important property of intact islets and reasonably accounts for the low peak on the normal glucose tolerance curve.

Evidence that the first step in functional deterioration of the beta cell is *delayed rate of response* to a rising blood sugar level is suggested by the shape of the glucose tolerance curve in even milder diabetics than those included in the present study. Seltzer, Fajans and Conn[16] described patients with symptomatic post-alimentary hypoglycemia as an early manifestation of diabetes mellitus. Their glucose tolerance test was characterized by normal fasting blood sugar, an early hyperglycemic plateau, and a late drop to hypoglycemic levels. The syndrome was thought to reflect sluggish initial release of insulin by islets which ultimately responded to the prolonged hyperglycemia with excessive insulogenesis. When diabetes progressed in these individuals, an upward shift of the glucose tolerance curve eliminated postprandial hypoglycemia at the cost of imposing fasting hyperglycemia, and the patient advanced into the present "tolbutamide-responsive-adult-diabetic" classification. In early diabetes, therefore, initial blunting of the normal swift response to a rising blood sugar level seems to activate a self-perpetuating cycle of (a) first, intermittent, postprandial hyperglycemia; which causes (b) prolonged, excessive stimulation of beta cells; which (c) progressively expends insulin-secreting reserve; until finally (d) *fasting* hyperglycemia supervenes, signifying round-the-clock flogging of an already weakened insulogenic mechanism.

In the present study, the mild fasting hyperglycemia of tolbutamide-responsive adult diabetics was associated with slightly above-normal levels of fasting plasma insulin activity. This suggestion of partial, albeit inadequate, response to a chronic betacytotropic stimulus was even better demonstrated in Vallance-Owen's group of elderly diabetics,[9] whose mean fasting insulin activity was 193 micro-units of insulin per ml. of plasma compared to 70 micro-units per ml. in normal subjects. In further parallel to our tolbutamide-responders, Vallance-Owen's patients also showed subnormal enhancement of circulating insulin activity after oral glucose, their mean increment being 219 micro-units of insulin per ml. of plasma compared to a rise of 333 micro-units per ml. in normal subjects.

The sequelae of unremitting hyperglycemia have been documented experimentally, and were also evidenced in our two severest diabetic groups. Barron[4] degranulated beta cells of normal dogs by administering glucose continuously by vein for only four to nine days; and Dohan and Lukens[5] produced permanent diabetes in the intact cat by sustaining hyperglycemia for forty days with intraperitoneally injected glucose. In our adult diabetics who did not respond to tolbutamide, severe depletion of insulin-secreting capacity was indicated both by subnormal insulin activity during fasting, and its minimal enhancement after glucose loading despite a twice-normal rise of blood sugar. Finally, with both morphology and function of beta cells obliterated in advanced juvenile diabetes,[17] neither fasting nor postglucose insulin activity was anticipated or encountered.

In contrast to their facile correlation with the thesis of primary beta-cell failure, the present findings do not fit well with Mirsky's concept[18] that ". . . an increase in the rate of destruction of insulin by the liver and other tissues, rather than a decrease in the production of insulin by the pancreas, is responsible for the clinical syndrome in the majority of patients with diabetes mellitus." Insulin is presumably inactivated mainly by the enzyme system, insulinase. If insulin secretion keeps pace with magnitude of glycemic stimulus, however, circulating insulin activity in more hyperglycemic tolbutamide-nonresponsive diabetics should be higher, instead of lower, than in the milder tolbutamide-responsive patients. This conclusion obtains whether the liver or peripheral tissues be favored as the major quantitative site of insulinase activity. The hypothesis of primary peripheral destruction of insulin thus seems to require the corollary that the resulting hyperglycemic state begins to deplete insulogenic reserve fairly early in the clinical course of the disease. In brief, even when one seeks the etiological locus of diabetes mellitus outside the beta cell, one is returned to that cell in short order.

SUMMARY

Plasma insulin activity during fasting and one hour after ingestion of 100 gm. of glucose was determined in twelve normal subjects, nine tolbutamide-responsive

adult diabetics, six tolbutamide-nonresponsive adult diabetics, and five juvenile diabetics. An "insulogenic index" relating enhancement of circulating insulin activity to magnitude of glycemic stimulus, enabled comparison of the insulin-secreting capacity of respective groups.

Average fasting blood glucose levels of diabetic patients were proportional to clinical severity of disease. After glucose loading, increments of blood sugar concentration were two or three times greater in diabetics than in control subjects.

Mean fasting plasma insulin activity was 59 micro-units of effective insulin concentration per ml. of plasma in normal subjects; 103 micro-units per ml. in tolbutamide-responsive adult diabetics; 36 micro-units per ml. in tolbutamide-nonresponsive adult diabetics; and 1 micro-unit per ml. in juvenile diabetics. One hour after glucose, respective values were 573 micro-units per ml. in normals; 453 micro-units per ml. in tolbutamide-responders; 77 micro-units per ml. in tolbutamide-non-responders; and 3 micro-units per ml. in juvenile diabetics.

Compared to an insulogenic index of 100 per cent for normal subjects, corresponding indexes were 25 per cent for tolbutamide-responsive diabetics, 4 per cent for tolbutamide-nonresponsive patients, and 0.1 per cent for the juvenile group.

The data indicate that total insulogenic reserve is sharply reduced in diabetes mellitus even when apparent impairment of carbohydrate tolerance is minimal, that it drops progressively as the clinical condition worsens, and approaches zero in the "total diabetic."

SUMMARIO IN INTERLINGUA

Le Activitate de Insulina in le Plasma post Cargation a Glucosa: Un Indice del Reserva Insulinogene in Humanos Normal e Diabetic

Le activitate de insulina in le plasma esseva determinate in stato jejun e un hora post le ingestion de 100 g de glucosa in dece-duo subjectos normal, novem diabeticos adulte respondente a tolbutamido, sex diabeticos adulte non respondente a tolbutamido, e cinque diabeticos juvenil. Un "indice insulinogene," destinate a establir un relation inter le promotion del activitate de insulina circulante e le magnitude del stimulo glycemic, permitteva un comparation del capacitate insulino-secretori in le varie gruppos de patientes.

Le valores medie del nivellos de glucosa sanguinee in stato jejun esseva proportional al severitate clinic del morbo in le caso del patientes con diabete. Post le cargation con glucosa, le augmentos in le concentration de sucro sanguinee esseva duo o tres vices plus grande in le diabeticos que in le subjectos de controlo.

Le valor medie del activitate de insulina in le plasma in stato jejun esseva 59 micro-unitates de concentration de insulina effective per ml de plasma in subjectos normal, 103 in diabeticos adulte respondente a tolbutamido, 36 in diabeticos adulte non respondente a tolbutamido, e 1 in diabeticos juvenil. Un hora post le administration de glucosa, le cifras correspondente esseva 573 in normales, 453 in responsores a tolbutamido, 77 in non-responsores a tolbutamido, e 3 in diabeticos juvenil.

Basate super le comparation con un indice insulinogene de 100 pro cento in subjectos normal, le correspondente indices esseva 25 pro cento pro diabeticos adulte respondente a tolbutamido, 4 pro cento pro diabeticos adulte non respondente a tolbutamido, e 0,1 pro cento pro diabeticos juvenil.

Le datos indica que le reserva insulinogene total es marcatemente reducite in patientes con diabete mellite, mesmo quando le apparente disturbation del tolerantia pro hydratos de carbon es minimal, que ille reserva declina progressivemente durante que le condition clinic del patiente deveni pejor, e que illo approcha zero in le caso del "diabetico total."

ACKNOWLEDGMENT

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Of the various theories concerned with protein chemistry, our results supported only the classical peptide hypothesis of Hofmeister and Fischer. The fact that all our results could be explained on this theory added further proof, if any were necessary, of its validity. The results also showed that proteins are definite chemical substances possessing a unique structure in which each position in the chain is occupied by one, and only one, amino acid residue.

Examination of the sequences of the two chains reveals no evidence of periodicity of any kind, nor does there seem to be any basic principle which determines the arrangement of the residues. They seem to be put together in an order that is random, but nevertheless unique and most significant, since on it must depend the important physiological action of the hormone.

BIOLOGICAL ACTIVITY AND CHEMICAL STRUCTURE

As yet little is known about the relationship of the physiological action of insulin to its chemical structure. One approach to this problem was to study the insulins from different animal species. Since all insulins show the same activity, it could be concluded that differences would be found only in parts of the molecule that were not important for activity.

All the results given above were obtained on insulin from cattle. When insulins from four other species were studied by essentially the same methods, it was found that the whole of the B chain was identical in all species, and the only differences that were found in the three amino acids contained within the disulfide ring of the A chain, which in the cattle are

Ala-Ser-Val

and in the other species are as follows:

Pig, Thr-Ser-Ileu
Sheep, Ala-Gly-Val
Horse, Thr-Gly-Ileu
Whale, Thr-Ser-Ileu

These results suggest that the exact structure of the residues in this position is not important for biological activity, but it does not follow that the whole of the rest of the molecule is important.

The determination of the structure of insulin clearly opens up the way to similar studies on other proteins, and already such studies are going on in a number of laboratories. These studies are aimed at determining the exact chemical structure of the many proteins that go to make up living matter and hence at understanding how these proteins perform their specific functions on which the processes of life depend. One may also hope that studies on proteins may reveal changes that take place in disease, and that our efforts may be of more practical use to humanity.

From "Chemistry of Insulin" by F. Sanger, in *Science*, May 15, 1959, Vol. 129, No. 3359. Reprinted with permission of the Nobel Foundation.

The Effect of Insulin-binding Serum Globulin on Insulin Requirement

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An insulin-binding globulin has been demonstrated in the serum of most patients previously treated with insulin.[1] Although this insulin-binding globulin may alter the biologic activity of insulin,[2-6] it has not been possible to demonstrate a direct correlation between the insulin requirement of diabetic patients and the insulin-binding capacity of their sera.[2,4,5] Thus, the clinical implications of the insulin-binding globulin are not apparent.

Characteristics of the binding globulin other than its total binding capacity for insulin may account for the lack of correlation with insulin requirements previously reported. Firstly, the reversibility and degree of dissociation between the bound and unbound insulin may influence the clinical response to insulin. Secondly, the extent to which the binding globulin has been saturated may alter insulin availability. Thirdly, the rate at which the binding globulin is produced may change during the course of diabetes mellitus. Clinical observations that can be related to alterations in these characteristics of the insulin-binding globulin will be described.

METHODS

Insulin labeled with iodine 131* (insulin I^{131}) was given to patients by intravenous injection in dosages varying from 0.3 to 3.6 U of insulin and 75 to 150 microcuries of iodine^{131}. Venous blood samples were obtained following injection at the intervals shown in the experimental detail. Serum was partitioned by precipitation with tricholoroacetic acid (TCA) into TCA soluble and TCA insoluble portions on 0.1 ml. of each serum sample. The intact insulin contained in each serum was separated by electrophoretic-convection flow on paper.[1,6] All analyses were carried out in duplicate. Strips used in electrophoresis were stained with 5 per cent bromphenol blue to differentiate the protein fractions. All samples were counted in a shielded well-type scintillation counter connected through a linear pre-amplifier to a scaler. The monitoring apparatus had a sensitivity of 10,000 counts per minute per 0.01 millicuries of radioiodine over a background of 180 counts per minute. Counts were carried out to sufficient numbers as to reduce the error of counting to less than 3 per cent. The quantity of radioactivity injected into each patient was determined by the "serum phantom" technic previously described.[7]

Propylthiouracil, 100 mg., and potassium iodide, 0.5 gm., were given to each subject every eight hours for two days prior to the study and continued for five days after injection of the radioactive material to prevent thyroidal accumulation of iodine^{131} and to promote excretion of the degradation products of the radioinsulin. Studies of excretion revealed that approximately 85 per cent of the injected radioactivity could be recovered in the urine in seventy-two hours.

In these studies the radioactivity in each of the partitions has been expressed as the percentile of the injected material contained in the total body plasma at each time interval. Plasma volume was assumed to be 5 per cent of the body weight. This method of calculation has been used in order to allow correlation in studies of different subjects. The iodine space, previously determined as being approximately 25 per cent of total body weight at equilibrium,[8] was used to calculate the free iodine^{131} contained in the body at specific times. The sum of the iodine^{131} excreted in the urine and the iodine^{131} contained in the iodine space is a measure of the degradation of the injected radioinsulin for any given time interval.

RESULTS

The clinical characteristics of the patients selected for study are shown in table 1. Patients I, II, III, who had diabetes mellitus, had optimal insulin dosages of 190, 140 and 80 units per day respectively. At the time of the initial study they were on inadequate quantities of insulin and under poor diabetic control. The fourth patient, also a diabetic, had received insulin for twenty

* Obtained from Abbott Laboratories.

Presented in part at the Nineteenth Annual Meeting of the American Diabetes Association in Atlantic City on June 7, 1959, under the title "Clinical Studies of Insulin I^{131} Partitions in Diabetic Patients."

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TABLE 1

Characteristics of patients studied

No.	Patient	Age	Sex	Race	Duration of insulin therapy (yr.)	Insulin dose at test (U./day)	Optimal insulin (U./day)	Clinical status at test
I	W.S.	34	M	N	1.8	45	190	Poor control
II	E.S.	60	F	N	6.0	90	140	Poor control
III	B.B.	40	F	N	3.0	60	80	Poor control
IV	B.H.	39	M	N	20.0	32	32	Brittle, early onset
V	A.M.	74	M	N	0	0	10	Mild diabetes, no insulin
VI	M.V.	34	F	N	0	40	40	Mild diabetes, insulin, four days
VII	J.D.	50	M	W	0	0	0	No diabetes, functional hypoglycemia

years and could be described as insulin sensitive; he had wide variations in the level of blood glucose typical of the "brittle" diabetic. Two patients, V and VI, had mild diabetes of several years' duration; patient V had not received insulin, and patient VI had received insulin for only four days. Patient VII, without diabetes mellitus, had been hospitalized for hypoglycemia as a consequence of previous gastric surgery.

These patients were given radioinsulin by intravenous injection and the percentage of the injected insulin that is contained in the total body plasma of each patient at intervals is shown in figure 1. These patients retained from 79 to 3 per cent of the radioinsulin at 120 minutes following injection. The percentage of the injected insulin that is retained by the insulin treated patients (I-IV) is in the same order as their optimal insulin requirements. Patients not previously treated with insulin retained little radioinsulin in their serum after 120 minutes.

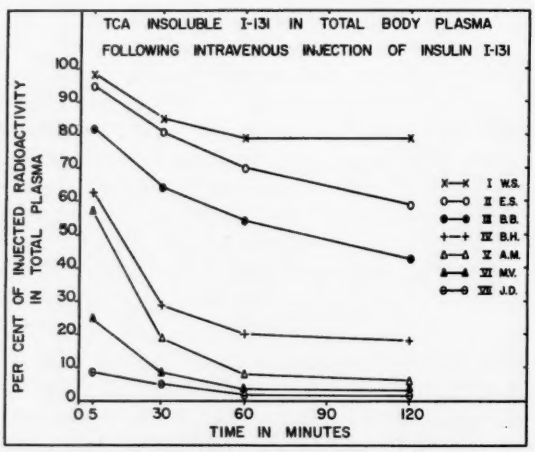

FIG. 1. The per cent of injected insulin I^{131} contained in the total body plasma as the TCA insoluble I^{131} has been determined in each patient. Compare with table 1 for the clinical characteristics of each patient.

Insulin bound to the insulin-binding protein is protected from degradation as shown in table 2. There is an inverse relationship between insulin retention and insulin degradation. The patients showing the highest retention of insulin in the total body plasma show the least degradation of insulin at sixty and 120 minutes following injection. The patients not previously treated with insulin degrade the injected insulin rapidly.

TABLE 2

Correlation between the radioactivity in the TCA precipitate and degradation of insulin

Patient		Time (in minutes) after intravenous insulin I^{131}: Per cent in TCA precipitate 60 min.	120 min.	Per cent of insulin degradation 60 min.	120 min.
I	W.S.	79	79	9	14
II	E.S.	70	59	20	27
III	B.B.	54	43	23	32
IV	B.H.	20	17	45	50
V	A.M.	9	8	56	—
VI	M.V.	4	3	58	62
VII	J.D.	4	3	67	—

It seemed likely that an increased proportion of injected insulin would remain unbound and become available for peripheral use if it were possible to sat-

urate the insulin-binding globulin. In figure 2 is shown the amount of radioactivity contained in the body plasma on three occasions in Patient I, W.S., while receiving 45, 90 or 190 units, respectively, of insulin by daily injection. Both the total insulin, as the TCA precipitate, and the free insulin, separated promptly by convection flow, are shown. At the time of the first test, the patient was receiving 45 units of insulin by daily injection and he retained 79 per cent of the injected radioinsulin at sixty and 120 minutes. At the time of the second test, eight days later, his insulin had been advanced by increments to 90 units per day and he retained 71 and 60 per cent of the radioinsulin at sixty and 120 minutes, respectively. The third test was completed ten days following the second, after the optimal dosage of insulin had been established at 190 units per day. On this occasion he retained only 48 and 43 per cent of the radioinsulin at sixty and 120 minutes. The amount of injected insulin remaining in the unbound form at five minutes after injection increased from 2.2 per cent in test I to 11.1 per cent in test III, as more of the binding protein became saturated with the insulin given by daily injection. An inverse relationship between the binding and degradation of insulin was also noted in these experiments. Degradation of the radioinsulin at 120 minutes following injection increased from 14 to 22 and 33 per cent, respectively, in the three experiments.

From the results shown in figure 2, it is clear that some insulin remains in the free form despite the presence of the insulin-binding globulin. Since free insulin is degraded quickly in vivo,[7] this suggests that some degree of dissociation between the bound and unbound insulin occurred in these patients, as has been shown previously in vitro.[9] The degree of dissociation can be expressed as the percentage of the total insulin that is found in the unbound form. Measurements of total insulin and free insulin were made on two occasions in Patient II, E.S., and in Patient III, B.B., without insulin loading. The results of these experiments and of the previous studies in Patient I, W.S., are shown in table 3. A relatively constant portion of the total insulin is in the free form throughout the period of study in patients E.S. and B.B. and in tests I and II of W.S. This is consistent with the hypothesis that there is a dissociation constant between the bound and unbound insulin by which insulin becomes available for peripheral use. The proportion of total insulin in the unbound form was greatly increased by the saturation of the binding globulin in Patient W.S. (test III).

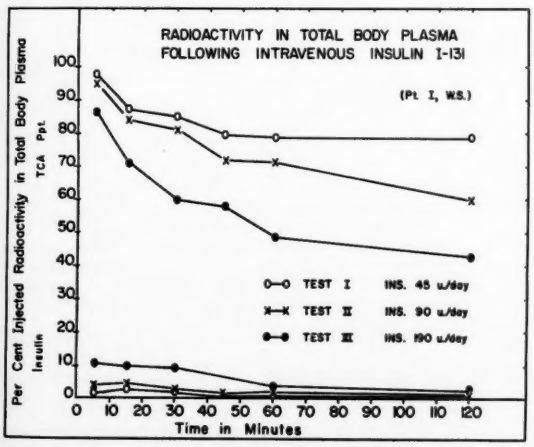

FIG. 2. The per cent of injected insulin I^{131} contained in total body plasma has been measured as the bound insulin (TCA precipitate) and unbound insulin (separated by electrophoresis). Results in one individual are shown at three dosages of insulin. Less insulin is bound and more insulin remains in the unbound form as the insulin-binding globulin becomes saturated by the increased insulin dosage.

TABLE 3

The per cent of total insulin found in unbound form in serum obtained at intervals after intravenous administration of insulin I^{131}

Patient	Test	Minutes after insulin I^{131}			
		5	30	60	120
II E.S.	I	3.3	2.7	2.3	2.5
	II	2.2	2.4	2.2	2.2
III B.B.	I	4.4	3.9	3.2	4.1
I W.S.	I	2.1	1.2	0.9	0.9
	II	3.3	2.2	1.7	1.3
	III	17.6	13.0	5.4	3.4

Although raising the dosage of insulin in patients whose serum contained large quantities of insulin-binding globulin allows a larger proportion of insulin to become available for peripheral use, increasing the dosage may also act as an added stimulus to further production of the insulin-binding globulin. The sera of patients previously treated with insulin were diluted serially with isotonic saline. Each serum dilution was placed with unlabeled insulin in combination with insulin I^{131} in a total concentration of 0.2 units of insulin per ml. The mixture of serum and insulin was allowed to equilibrate over a sixty-minute period. The unbound and bound insulin were separated in each dilution by means of the electrophoretic-convection technic and the radioactivity in each portion was determined as previously stated. The insulin-binding protein complex travels with the gamma globulin when separated by this

technic[3] although it may move more rapidly in other systems.[10] The results were expressed as the percentile of the total insulin I^{131} bound to the serum globulin. The serum of each patient was tested again for the insulin-binding capacity after one to three months on their optimal insulin dosages. The results of the first test are shown in figure 3. The serum showing the highest insulin-binding in all dilutions was obtained from the patient requiring 190 units of insulin per day (I, W.S.). The least insulin-binding was obtained in the serum of Patient IV, B.H. Patients II, E.S., and III, B.B., had binding capacities that were intermediate between these two parameters.

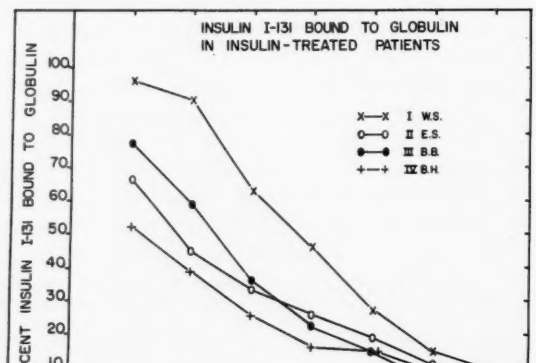

FIG. 3. The per cent of insulin I^{131} bound to globulin in serial dilutions of the sera of insulin treated patients. The insulin-binding properties shown here, in vitro, are of the same order as the insulin I^{131} retention in total body plasma shown in figure 1.

The insulin dosage of Patient B.H. remained unchanged and no change in the insulin-binding globulin was found after three months of continued insulin therapy. An increase in the amount of insulin-binding globulin was noted, however, in patients E.S. and B.B., after three months and one month, respectively, on the increased dosage of insulin (figure 4). The results of the tests on Patient I are shown in figure 5. There was no change in the quantity of insulin-binding globulin before and two months after the institution of the optimal insulin dosage. Methyl prednisolone (Medrol) therapy, 16 mg. per day, was instituted for one month prior to a third test. As shown in figure 5, there was a decrease in the insulin-binding globulin in all dilutions following institution of steroid therapy. The patient remained well controlled throughout the initial period of adrenal steroid therapy. Subsequently, while

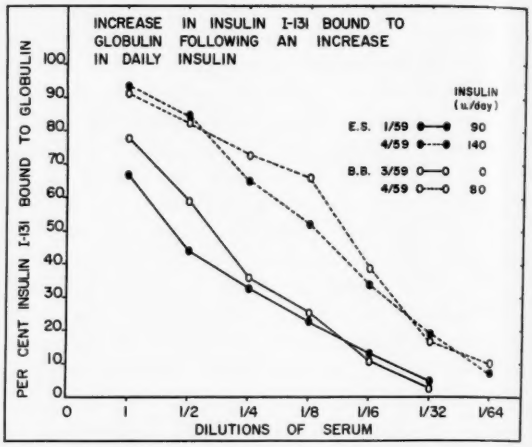

FIG. 4. The per cent of insulin I^{131} bound to globulin in serial dilution of the sera of patients II, E.S., and III, B.B., before, and after the increase in daily insulin dosage. There is an increase in the total binding capacity of both sera following the change in insulin therapy.

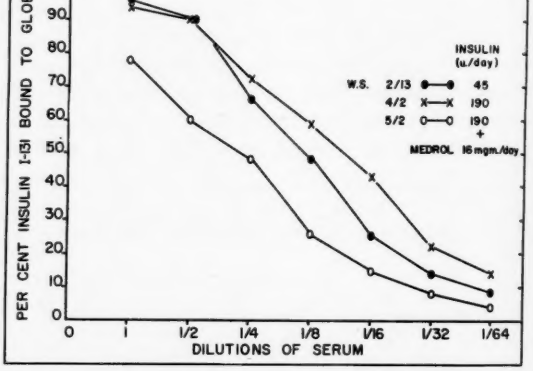

FIG. 5. The per cent of insulin I^{131} bound to globulin in serial dilutions of the sera of patient I, W.S. There was no change in the total binding capacity after approximately two months on the increased dosage of daily insulin. The binding capacity was decreased one month following the institution of methyl prednisolone (Medrol), 16 mg./day.

continuing on this therapy, his insulin dosage was reduced to 160 units per day following several mild episodes of sweating. These were interpreted as being due to hypoglycemia although blood sugar determinations were not obtained at the times of the symptoms.

DISCUSSION

Previous investigators have shown a good correlation, in general, between the insulin-binding and insulin-

inhibiting effects of sera of patients previously treated with insulin.[2,4,11] Kalant and his associates[5] reported that patients previously treated with insulin for six months or longer, had a decrease in the effect of insulin during a glucose-insulin tolerance test. Failure to find a good correlation between the daily insulin requirements of insulin-treated patients and the insulin-binding capacity of their sera has cast doubt, however, on the clinical significance of insulin-binding globulin.[2,4,5] Many of the patients studied for this possible correlation had complications of their illness that would be expected to raise insulin requirements without necessarily affecting the binding capacity. This possibility cannot be excluded from studies in which the clinical details of the patients have been omitted. In addition, insulin antagonists of other types may possibly obscure the relationship.[11]

The studies on the effect of the saturation of insulin-binding globulin by increased dosages of daily insulin reported here, illustrate a further difficulty in demonstrating a *direct* correlation between insulin dosage and retention of radioinsulin. It was shown that as the insulin dosage is increased, insulin-binding globulin becomes saturated and the percentage of insulin retained in the body plasma decreases. The degree of saturation of the binding globulin appears to be related to the ratio between the insulin administered and the optimal dosage of insulin required. The ratio, in percentage, between the administered and optimal dosages of insulin in each patient studied has been tabulated in ascending order and correlated with retention and degradation of the radioinsulin at sixty and 120 minutes (table 4). When considered in this way, it can be seen that the percentage of radioinsulin degraded varies directly and the percentage of insulin retained varies inversely with the percentage of the optimal insulin dosage administered. Thus, it appears that the degree of saturation of the binding globulin rather than the insulin dosage is the crucial factor in the degree of retention or degradation of the radioinsulin.

TABLE 4

Comparison of degree of saturation of insulin-binding globulin with degradation and retention of radioinsulin in vivo

Patient	Test	Per cent saturation*	Per cent degradation 60 min.	Per cent degradation 120 min.	Per cent retention 60 min.	Per cent retention 120 min.
I W.S.	I	24	9	14	79	79
I W.S.	II	47	14	20	72	63
II E.S.	—	64	20	27	70	59
III B.B.	—	75	23	32	54	43
I W.S.	III	100	26	31	50	43
IV B.H.	—	100	45	50	20	17

*Per cent saturation $= \frac{\text{Insulin dosage at test}}{\text{Optimal insulin dosage}} \times 100$

See text for discussion.

Within the limitations of the procedure, it was possible to demonstrate that a relatively constant proportion of the injected insulin is present in the unbound form in patients with large quantities of binding globulin. The three studies in Patient I, W.S., show that more insulin is made available for peripheral use by the saturation of the binding globulin. Raising the daily dosage of the insulin in patients with insulin-binding globulin, increases the absolute quantity of available insulin, firstly, by a constant proportion of the increased dosage and secondly, as the binding globulin becomes saturated, by an increased proportion of the total insulin.

Patient IV, B.H., had early onset diabetes of the "brittle" type and had been treated with insulin for twenty years. This patient had less insulin-binding globulin than the other insulin-treated patients. There was no evidence that the total insulin-binding globulin of his serum changed over the period of several months. During this period, he continued to have wide fluctuations in the level of the blood glucose.

It is possible that the presence or absence of a large plasma insulin "depot" in the form of insulin reversibly bound to globulin may be the fundamental difference between "brittle" patients and patients less sensitive to insulin changes. The latter group of patients may be sustained between injections of exogenous insulin either by small amounts of endogenous insulin or by the dissociation of free insulin from the insulin-binding protein. Patients with "brittle" diabetes, on the other hand, are lacking in endogenous insulin stores,[12,13] and may lack the sustained supply of insulin afforded by dissociation from the insulin-binding globulin. This interesting possibility requires further study.

There is some question as to whether or not estimates of the total quantity of insulin-binding globulin in sera obtained at different times and under different circumstances are comparable. On the basis of the dissociation of the bound and unbound insulin in vitro,[9] it has been assumed that the radioinsulin serves as an accurate measure of the total binding capacity of the serum providing sufficient time is allowed to achieve a steady state after mixing of the insulin and serum. Berson and Yalow[9] have reported that a rapidly dissociating and a slowly dissociating insulin-binding com-

plex can be demonstrated in the sera of insulin treated patients. These insulin-binding globulins are possibly related to the administration of insulins from two different animal species. A number of dissociation curves between the two extremes are shown, however, and these curves would also be consistent with the progressive dissociation of an insulin-protein complex that becomes larger with time. Dilution studies of the sera would not conclusively differentiate between these two possibilities.[9] In either case, it is questionable whether a tightly bound single complex or a large aggregate would be labeled by the radioinsulin added to the serum. Studies of the serum of patient I, W.S., before and after insulin loading, show no change in the binding capacity of the serum. This is in agreement with the assumption that the total binding capacity of the serum, rather than the unsaturated binding sites, are measured by the technics used.

High levels of binding globulin may not be accurately measured by the use of a single ratio between the insulin and the binding globulin. Under these circumstances, it will not be possible to differentiate between any of the sera that bind most of the available insulin. By using serial dilutions of the sera, with a constant quantity of insulin labeled with I^{131}, however, measurement of high levels of the binding globulin is possible. This is illustrated by the studies on the sera of Patient W.S. There was little change in the quantity of insulin-binding globulin following the increase in the daily dosage of insulin. After the addition of corticoid therapy, there was a reduction in the total binding capacity from approximately 400 units of insulin to approximately 160 units of insulin per liter of body plasma. Subsequently, as the corticoid was continued, there was a modest reduction in the daily insulin requirement.

Field[4] has reported the effect of ACTH on the insulin requirement of a patient with chronic insulin resistance. The daily insulin dosage was 375 units before and 30 units after ACTH therapy. The ability of one milliliter of this patient's undiluted serum to bind 0.2 units of insulin labeled with I^{131} remained the same before and after the ACTH therapy. However, it is difficult to be certain that total binding capacity was not reduced under these circumstances without further dilution studies.

There was an increase in the total insulin-binding capacity in the sera of patients II, E.S., and III, B.B., after the daily insulin dosage was increased. No change in clinical response to the daily dosage of insulin was noted as a result of the increase in the total binding capacity of their sera. Studies of insulin I^{131} retention and degradation were not carried out at the time of this increase in the binding globulin.

The present studies indicate that there is some correlation between the level of the insulin-binding globulin and the insulin requirements of diabetic patients. Calculation of the total quantity of insulin that may be bound in vivo, on the basis of the total binding capacity in vitro and the use of this figure as a basis for the daily insulin dosage, may not be realistic, however. Thus, Patient W.S. required 190 units of insulin per day but bound approximately 400 units of insulin per liter of plasma. The degree of saturation as the result of previous insulin, the rate of production, or turnover of the binding globulin and the degree of dissociation between unbound and bound insulin appear to influence insulin requirements and the total binding capacity.

SUMMARY

An insulin-binding globulin in the sera of patients previously treated with insulin has been shown to have an effect on their insulin requirements. The amount of insulin that is available for peripheral use is inversely proportional to the quantity of unsaturated binding protein of the serum. Some injected insulin is found in the unbound form despite high levels of insulin-binding globulin, however, presumably by dissociation from the insulin bound to globulin. Increasing the daily dosage of insulin saturates the binding globulin and allows a greater proportion of the injected insulin to remain in the unbound or free form. The increased insulin dosage may stimulate further production of the insulin-binding globulin. Administration of methyl prednisolone decreased the total binding capacity of the serum in one patient tested. Thus, the degree of saturation of the binding globulin as a result of previous insulin therapy, the degree of dissociation between the bound and unbound insulin, the rate of production of insulin-binding globulin in addition to the total insulin-binding capacity of the serum, appear to influence insulin requirements in diabetic patients previously treated with insulin.

SUMMARIO IN INTERLINGUA

Le Effecto de Globulina Insulino-Ligante del Sero Super le Requirimento de Insulina

Esseva monstrate que un globulina insulino-ligante in le seros de patientes previemente tractate con insulina ha un effecto super lor requirimento de insulina. Le quantitate de insulina disponibile pro uso peripheric es inversemente proportional al quantitate de non saturate globulina insulino-ligante in le sero. Tamen, un

certe quantitate del injicite insulina es trovate in forma non-ligate in despecto del presentia de alte nivellos de globulina insulino-ligante, presumitemente in consequentia de dissociation ab le insulina ligate a globulina. Le augmentation del dosage diurne de insulina resulta in le saturation del globulina ligante e permitte a un plus grande proportion del injicite insulina remaner in le forma non-ligate o libere. Le augmentate dosage de insulina pote stimular additionalmente le production de globulina insulino-ligante. In un patiente il esseva trovate que le administration de un steroide adrenal reduceva le total capacitate ligatori del sero. Assi il pare que le requirimento de insulina in diabeticos previemente tractate con insulina depende—a parte le total capacitate insulino-ligante del sero—de tres factores, i.e. (1) le grado de saturation del globulina ligante que es un resultato del previe therapia a insulina, (2) le grado de dissociation inter le ligate e le non ligate insulina, e (3) le production de globulina insulino-ligante.

ACKNOWLEDGMENT

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In 1948, D. E. Green proposed that not only the electron-transfer mechanism but also the mechanisms for citric cycle oxidation, oxidative phosphorylation, and fatty acid oxidation were associated with one and the same particulate complex, which was called the "cyclophorase system." It was postulated that the large number of enzymes and all the auxiliary coenzymes which implemented each of these sequential processes were linked together in a very precise manner within the structural framework of a giant macromolecule. The complex was assumed to be the unit of enzymatic action in the same sense that the polyfunctional pyruvic dehydrogenase has been defined previously as a unit of enzyme action. It is unnecessary in this paper to summarize the various lines of evidence which led to the cyclophorase concept. To be sure, the evidence first presented was more circumstantial than conclusive. It was probably not so much the incomplete nature of the evidence that engendered widespread skepticism as it was the strangeness of the concept and the multiplicity of reactions encompassed by the cyclophorase system. But the groundwork had been laid for the more sympathetic reception which came when Potter and Schneider and, independently, Lehninger and Kennedy established that the mitochondrion did, in fact, carry out precisely the sequences postulated for the cyclophorase system.

From an article by David E. Green and Johan Jarnefelt in *Perspectives in Biology and Medicine*, Vol. 2, page 163, 1959.

Influence of Dietary Fructose on Glucose Tolerance in Man

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Hill, Baker and Chaikoff[1] have reported that rats that had been fasted for one day and fed a diet in which fructose was the only carbohydrate for the next three days showed almost as great a loss of tolerance for orally administered glucose as did rats that were fasted for four days. These two groups of rats were compared with a group that were fasted for one day and fed a diet in which glucose was the only carbohydrate for the next three days. The results of studies of the utilization of C^{14}-labeled glucose, fructose, and acetate in tissue slices from the glucose-fed and fructose-fed animals suggested that liver glucokinase activity was decreased in the fructose-fed rats. Glucose levels in portal vein blood were found to be lower after fructose ingestion than after glucose ingestion. The authors suggested that the hepatic glucokinase system became adapted to dietary glucose and required the presence of frequent high concentrations of glucose in the portal vein blood to maintain its optimal activity. In the rat it appeared that glucose was the dietary carbohydrate specifically required to maintain the activity of the mechanisms for the disposal of administered glucose. Comparable experiments have been performed in the dog by Hill and Chaikoff[2] who demonstrated a diminished glucose tolerance in animals that were fed a fructose-containing diet for seven days.

These findings were interesting but unexpected. Since it had been shown that fructose could be converted to glucose in intestinal,[3-5] hepatic,[6,7] and renal[8-10] tissue, marked effects of glucose deprivation would not have been anticipated in a fructose-fed animal. It had also been reported that fructose administration restored glucose tolerance to normal in a fasted human subject.[11] The phenomenon demonstrated by Hill and others in the rat and dog might explain the loss of the initial advantage of fructose over glucose which was described in the depancreatized dog after prolonged fructose feeding.[12] If this phenomenon occurred in man, it might account for the reported loss of advantage of fructose after prolonged feeding to diabetic patients.[13] It would also suggest a mechanism of starvation diabetes in man.

With these considerations in mind, the present studies were designed to determine whether or not the ingestion of a diet whose sole carbohydrate was fructose would alter oral or intravenous glucose tolerance in man.

METHODS

The composition of the experimental diets, whose sole carbohydrate was either glucose or fructose,* is listed in table 1. Both diets contained approximately 95 gm. of protein, 75 gm. of fat, 250 gm. of carbohydrate, and a total of 2,055 calories. Their composition was exactly the same except for the different hexoses. One fifth of the meat and butter was eaten at breakfast, two fifths at lunch, and two fifths at supper. One fourth of the hexose was usually ingested with each of the three meals and a similar amount at bedtime. In addition to the diet, one multivitamin capsule† was taken each day.

The subjects for these studies were four healthy male physicians whose ages ranged from twenty-nine to thirty-four years. Three types of studies were performed:

(1) For a period of three days each subject ate the glucose diet. On the morning of the fourth day an intravenous glucose tolerance test was performed. For the next several days the subject consumed his usual mixed diet‡ in order to insure the return of his nutritional

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*The fructose used in these studies was supplied by Mead Johnson and Company.

†Avicaps were furnished by Burroughs Wellcome and Company.

‡The term "mixed diet" describes a diet composed of foods one would expect to find in the average American home or in the usual hospital cafeteria. Such a diet contains carbohydrate in several forms, in contrast to the experimental diets used in this study.

TABLE 1

Daily composition of experimental diets

Fructose diet (gm.)		Glucose diet (gm.)	
Ground round steak	500	Ground round steak	500
Butter	20	Butter	20
Fructose*	250†	Glucose*	250†

*In the form of a 10 to 50 per cent aqueous solution.
†The diet of subject J.C. contained only 200 gm. of hexose preceding the intravenous glucose tolerance tests.

status to its usual level. He then ate the fructose diet for three days. On the morning of the fourth day the intravenous glucose tolerance test was repeated. In half of the subjects the order of the experimental diets was reversed and the fructose dietary period preceded the glucose period. On other occasions, three of the subjects ate a mixed diet whose carbohydrate content was 250 gm. daily; after periods of three days on this diet intravenous glucose tolerance tests were performed.

(2) Since the above studies involved only the maintenance of normal glucose tolerance by fructose feeding, another experiment was performed in subject J.C. to determine whether or not glucose tolerance could be restored to normal after impairment by fasting. A control intravenous glucose tolerance test was performed after the subject had been on a mixed diet containing 200 gm. of carbohydrate daily for three days; the test was repeated after a thirty-three-hour period of fasting. The test was performed a third time after one day of fasting and three days on the experimental diet containing 200 gm. of fructose daily.

(3) In order to simulate the conditions of the rat experiments of Hill and others more closely, two of the subjects fasted one day and ate the experimental glucose or fructose diet for the next three days; on the morning of the fifth day oral glucose tolerance tests were performed. After the subjects had been eating the usual mixed diet again for several days, the same experiment was repeated except that the other hexose was used as the sole dietary carbohydrate.

Glucose tolerance tests were performed with the subject resting in the recumbent position following an overnight fast. The intravenous tests were performed by administering one gram of glucose per kilogram of body weight in a 10 per cent solution in water during one hour; an infusion pump was used to insure a uniform rate of administration. Blood samples for glucose, pyruvic acid, and lactic acid determination were obtained through an indwelling needle in an antecubital vein, before and at thirty-minute intervals after the start of the infusion, for a total of three hours. For the oral tests, 100 gm. of glucose in 500 ml. of water were ingested within a five-minute period. Blood samples were obtained at the same intervals as for the intravenous tests. The blood was analyzed for glucose by the method of Somogyi, using Nelson's chromogenic reagent.[14] Pyruvic acid was determined by the method of Friedemann and Haugen[15] and the procedure of Barker and Summerson[16] was used for lactic acid analysis.

RESULTS

Clinical effects of diet. The subjects experienced up to four soft to watery bowel movements during the three-day periods of the experimental hexose diets; diarrhea was experienced in the course of both glucose and fructose diets but more frequently during the latter. It was not felt that the frequency or severity of the diarrhea was sufficient to result in any significant loss of nutrients. Weight losses during the three-day dietary periods varied from zero to 1.3 kg. The weight changes were apparently related to the caloric content of the diet, and were not influenced by changing the form of the dietary carbohydrate.

Intravenous glucose tolerance tests following three days of experimental diet. The results of these tests are contained in the upper section of table 2. In three of the four subjects glucose disappeared somewhat more rapidly from the blood following the fructose diet than after the glucose diet; in the fourth subject (M.M.), the reverse was true. The average glucose tolerance curves of the four subjects after the two different hexose diets are illustrated in figure 1. Under the conditions of these experiments, a diet whose sole carbohydrate was fructose did not produce a significant decrease in the rate of removal of intravenously administered glucose.

Intravenous glucose tolerance tests following three days of mixed diet containing 250 gm. of carbohydrate. The results of these tests are shown in the upper section of table 2. It can be seen that these curves were similar to those obtained following three days of the experimental hexose diets. Changing the dietary carbohydrate from that encountered in the usual mixed diet to the same quantity of a pure hexose did not alter the rate of removal of subsequently administered glucose.

Intravenous glucose tolerance tests following one day of fasting and three days of fructose diet. The results of these tests in subject J.C. are shown in the lower section of table 2. One day of starvation produced the expected decrease in the rate of removal of administered glucose, while fructose feeding for three days restored the glucose tolerance to the previous control level.

TABLE 2

Influence of previous diet on blood glucose, pyruvic acid, and lactic acid concentrations during intravenous glucose tolerance tests* in normal subjects

Subject	Wt. Kg.	Date	Carbohydrate in previous diet	Blood glucose (mg./100 ml.) Minutes after start of infusion							Blood pyruvic acid (mg./100 ml.) Minutes after start of infusion							Blood lactic acid (mg./100 ml.) Minutes after start of infusion						
				0	30	60	90	120	150	180	0	30	60	90	120	150	180	0	30	60	90	120	150	180
J.C.	61	10/9/54	Glucose	94	267	342	180	101	57	57	1.1	1.0	1.5	1.4	1.2	1.1	1.0	3.7	4.5	8.9	10.0	9.4	6.0	6.5
		9/27/54	Fructose	89	241	274	137	55	78	82	0.9	0.9	1.2	1.3	1.0	1.2	1.2	3.9	3.7	6.0	6.8	4.5	9.5	7.3
M.M.	69	11/6/54	Glucose	81	211	275	69	56	48	60	0.9	0.7	1.7	1.3	0.9	1.0	0.8	6.8	8.6	9.3	15.5	7.4	7.4	8.3
		1/18/55	Fructose	88	259	294	143	74	67	71	0.8	0.8	1.1	1.0	0.8	0.8	0.9	1.2	1.6	4.2	5.5	3.9	3.6	3.5
		11/2/54	Mixed	74	240	287	115	45	42	56	1.1	1.1	1.4	1.5	1.2	1.1	1.0	7.0	7.3	8.9	10.0	8.2	6.9	9.6
		1/13/55	Mixed	77	203	245	155	91	63	65	0.9	1.1	1.2	1.5	1.1	0.9	0.8	3.4	4.3	4.9	6.9	6.1	4.0	3.6
W.D.	80	12/3/54	Glucose	78	300	397	238	73	44	57	1.0	1.8	1.4	1.3	1.2	1.1	1.0	6.5	17.7	8.1	11.8	9.7	8.4	9.9
		12/20/54	Fructose	85	283	389	125	42	49	62	0.7	1.3	1.0	1.3	0.7	0.6	0.9	3.5	6.0	9.0	10.5	6.5	3.0	6.6
		11/29/54	Mixed	86	278	359	142	67	38	61	1.1	1.0	1.3	1.1	1.0	0.7	1.0	5.3	4.7	9.5	7.7	6.2	4.7	7.7
		12/16/54	Mixed	91	296	384	146	66	45	65	1.0	1.2	1.2	1.4	1.1	1.3	1.5	9.8	—	9.4	9.7	9.2	10.7	15.5
C.P.	96	2/19/55	Glucose	92	317	424	283	199	129	86	0.8	0.9	1.2	1.2	1.0	0.8	0.5	1.8	1.9	4.2	4.9	3.7	1.9	4.1
		2/11/55	Fructose	96	289	346	174	103	54	80	0.8	1.0	1.0	1.3	0.7	0.4	1.4	3.3	4.5	4.4	6.0	6.9	10.1	7.9
		2/5/55	Mixed	98	298	380	265	142	81	56	1.1	1.0	1.0	1.2	1.1	0.8	0.6	2.5	6.4	7.4	9.3	6.2	5.8	7.7
J.C.		6/13/57	Mixed	87	237	291	146	67	57	71	1.2	0.8	1.4	1.2	0.8	0.9	1.1	5.2	7.6	9.9	8.2	8.2	7.4	8.9
		10/10/57	None (fasting one day)	86	230	327	232	176	118	90	1.3	1.1	0.9	1.2	1.2	1.3	1.2	7.9	7.7	6.9	7.0	6.9	5.8	5.9
		10/22/57	Fructose†	82	234	238	115	83	55	73	1.0	1.0	1.2	1.3	1.3	1.0	1.1	5.6	6.4	8.2	9.9	9.2	7.6	8.1

*One gram of glucose per kilogram of body weight in sixty minutes.
†Preceded by one day of fasting.

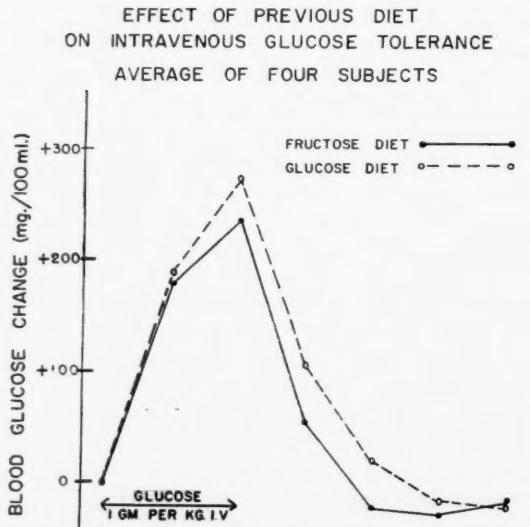

FIG. 1. Average of the intravenous glucose tolerance curves of the four subjects after three days on a diet whose sole carbohydrate was glucose and after three days on a diet whose sole carbohydrate was fructose. Blood sugar concentrations are plotted in terms of variation from the fasting level.

Oral glucose tolerance tests following one day of fasting and three days of experimental diet. The results of these tests are contained in table 3 and in figure 2. Although there was a marked difference between the heights of the blood sugar rises in the two individual subjects, the rate of removal of orally administered glucose from the blood was not impaired by the previous fructose diet in either case.

Changes in blood pyruvic acid and lactic acid during glucose tolerance tests. The usual rises in the concentrations of blood pyruvic acid and lactic acid were observed during the course of the glucose tolerance tests as shown in tables 2 and 3. Although these changes were variable in magnitude, the variations could not be correlated with a change in the form of carbohydrate in the previous diet.

DISCUSSION

The results of these studies suggest that in man dietary glucose* is not required to maintain the activity of the mechanisms for the removal of administered glucose from the blood. Glucose tolerance curves ob-

*The term "dietary glucose" as used here includes not only glucose but other forms of carbohydrate such as starch and glycogen which are converted to glucose in the gastrointestinal tract prior to absorption.

TABLE 3

Influence of fasting and subsequent fructose feeding on blood glucose, pyruvic acid, and lactic acid concentrations during oral glucose tolerance tests* in normal subjects

Subject	Date	Carbohydrate in previous diet	Blood glucose (mg./100 ml.) Minutes after start of infusion							Blood pyruvic acid (mg./100 ml.) Minutes after start of infusion							Blood lactic acid (mg./100 ml.) Minutes after start of infusion						
			0	30	60	90	120	150	180	0	30	60	90	120	150	180	0	30	60	90	120	150	180
J.C.	3/28/55	Glucose†	73	102	107	88	89	68	79	1.2	1.2	1.6	1.8	1.4	1.4	1.0	7.0	5.7	8.8	15.4	19.2	10.4	7.8
	3/7/55	Fructose†	87	104	99	95	90	85	87	0.5	0.6	1.0	0.8	0.7	0.9	1.0	2.4	3.6	8.3	8.4	9.2	9.5	8.1
W.D.	3/21/55	Glucose†	83	155	195	184	166	143	132	1.0	1.2	0.9	1.8	1.0	1.2	0.8	4.2	3.7	7.1	6.8	5.8	5.0	5.4
	4/11/55	Fructose†	92	183	214	176	137	108	81	0.8	1.0	1.2	1.4	1.2	1.2	1.0	7.8	7.1	10.5	13.2	11.7	9.7	10.5

*One hundred grams of glucose was employed as test dose.
†Preceded by one day of fasting.

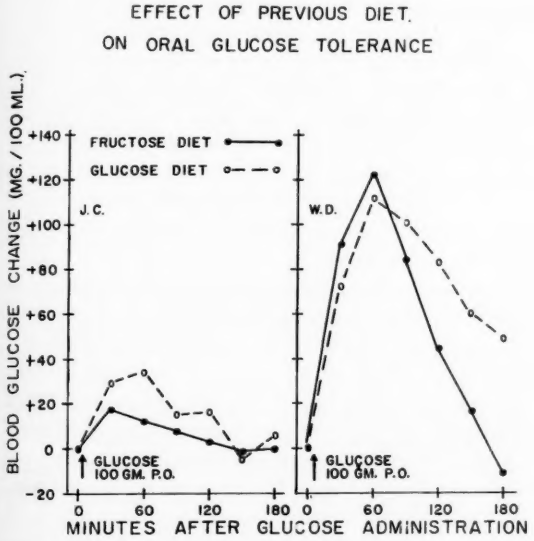

FIG. 2. Oral glucose tolerance curves of subjects J.C. and W.D. after one day of fasting and three days on a diet whose sole carbohydrate was glucose and after one day of fasting and three days on a diet whose sole carbohydrate was fructose. Blood sugar concentrations are plotted in terms of variation from the fasting level.

tained after the subjects had ingested a glucose-free (fructose) diet for three days were similar to those obtained following three days on a diet which was identical except that glucose was the sole carbohydrate. Although the results of these experiments should not be extrapolated to dietary periods longer than four days, one would expect that if a change in glucose tolerance were to occur it would be evident within this period of time, since a definite loss of tolerance has been observed after only two days of fasting or a carbohydrate-free diet.[17-18] The finding of Van Itallie and Shull[19] that the net tolerance for oral glucose was not impaired in normal human subjects after five days of fructose feeding lends additional suport to this conclusion.

However, our results do not exclude the possibility that in the absence of adequate dietary glucose, the activity of the glucose disposal mechanisms may be maintained by glucose formed endogenously from appropriate dietary precursors. This conclusion is based on the knowledge that in man a portion of administered fructose is converted to glucose.[20] The fact that glucose tolerance is more impaired by an antecedent low carbohydrate diet which is high in fat than by one with a high protein content[18] is consistent with this possibility, since dietary protein can furnish more "available carbohydrate" than can fat.

If the conversion of fructose to glucose is to explain the maintenance of normal glucose metabolism during a period when the sole dietary carbohydrate is fructose, one of two conditions must exist: (1) The mechanisms for glucose disposal must be relatively insensitive to a decrease in glucose supply; or (2) a large proportion of administered fructose must be converted to glucose. The studies of Himsworth furnish quantitative information regarding the relationship between dietary carbohydrate and the glucose tolerance curve.[21] This investigator found that as the amount of dietary carbohydrate was increased from 50 to about 150 gm. a marked improvement in glucose tolerance occurred; with further increase of carbohydrate from 150 to 350 gm., improvement in tolerance was relatively slight. These findings suggest that unless at least 150 gm. of glucose is formed from 250 gm. of dietary fructose, the glucose tolerance curve after the fructose diet should be higher than following a diet containing 250 gm. of glucose, assuming that glucose is essential for maintenance of the glucose disposal mechanism. Since precise information is lacking in regard to the portion of administered fructose which is converted to glucose in man, this question cannot be

resolved at the present time.

A study of portal anastomotic vein blood in a patient with Laennec's cirrhosis demonstrated that during the intestinal absorption of fructose less than one fifth of that hexose was converted to glucose.[20] Accordingly, a significantly lower concentration of glucose would occur in the portal blood after ingestion of fructose than after ingestion of an equal amount of glucose. One would therefore have expected a difference in glucose tolerance after the two different experimental diets if the portal blood glucose level in man were of critical significance in this regard, as Hill and his associates have postulated in the case of the rat.

In the present studies as in those of Hill and his associates in the rat and dog only the net tolerance for glucose was tested; no measurements were made of the handling of glucose by individual organs. Fructose feeding might impair the glucose disposal mechanisms of an organ whose quantitative contribution to the total body metabolism is so small that the change would not be detectable in the glucose tolerance curve. Alternatively, if fructose feeding enhanced the rate of glucose removal by one organ and impaired this process in another organ, the net tolerance for glucose might not be altered. Indeed Van Itallie and Shull[19] have concluded from epinephrine-glucose tolerance tests and measurements of the peripheral capillary-venous glucose differences that fructose feeding in man increases the rate of peripheral glucose removal while impairing the ability of the liver to take up glucose.

Three explanations may be proposed to account for divergent results in the rat and human studies:

(1) The mechanisms for glucose disposal may be dependent upon a continued supply of glucose for maintenance of their activity in the rat but not in man.

(2) The difference may be only quantitative in that mechanisms are less sensitive to a decreased supply of glucose in man. As mentioned earlier, a reduction in the total carbohydrate content of the diet produces a prompt decrease in glucose tolerance in both man and the rat, while dietary fructose maintains or restores the tolerance for glucose in man but not in the rat. It is difficult to explain these observations solely on the basis of a quantitative metabolic difference which might be associated with the different body sizes and rates of energy production of the two species.

(3) The glucose disposal mechanisms of the two species may be equally sensitive to changes in available glucose, but a smaller fraction of administered fructose may be converted to glucose in the rat. Relatively less glucose would therefore be available to maintain the disposal mechanisms in the fructose-fed rat as compared to the fructose-fed man. Sufficient data are not available either to exclude or confirm any one of these proposed explanations.

SUMMARY

1. Glucose tolerance tests were performed in normal human subjects who had been fed for three days a diet whose sole carbohydrate was either 200 or 250 gm. of glucose. The tests were repeated in the same subjects after three days on a diet whose sole carbohydrate was an equal quantity of fructose. Control tolerance tests were obtained after normal diets containing the same amount of carbohydrate in mixed form.

2. Fructose feeding for this period of time did not decrease the rate of removal of either orally or intravenously administered glucose. It restored glucose tolerance which had been impaired by fasting for one day.

3. In contrast to findings in the rat and dog, the specificity of glucose as the dietary carbohydrate responsible for maintaining the activity of the glucose disposal mechanisms was not demonstrated in man.

SUMMARIO IN INTERLINGUA

Le Influentia de Fructosa Dietari Super le Tolerantia pro Glucosa in Humanos

1. Tests de tolerantia pro glucosa esseva effectuate in normal subjectos human qui habeva recipite durante tres dies un dieta in que le sol carbohydrato esseva 200 o 250 g de glucosa. Le tests esseva repetite in le mesme subjectos post tres dies durante le quales le sol carbohydrato dietari esseva le mesme quantitate de fructosa. Valores de controlo esseva obtenite per tests de tolerantia post dietas normal continente le mesme quantitate de carbohydrate in forma miscite.

2. Le alimentation a fructosa durante le mentionate periodo de tempore non relentava le elimination de glucosa administrate per via oral o per via intravenose. Illo restaurava le tolerantia pro glucosa que habeva essite compromittite per un die de jejunamento.

3. Per contrasto con le constatationes in rattos e canes, le specificitate de glucosa como le carbohydrato responsabile pro mantener le activitate del mechanismos de elimination de glucosa non esseva demonstrate in humanos.

ACKNOWLEDGMENTS

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The function of insulin as a regulator of permeability of cell membranes is, at present, in the foreground of attention. Nevertheless, experiments such as those reported in the lucid paper by Kipnis and Cori on the change of the temperature coefficient for pentose transfer into diaphragm with insulin addition indicate a possible shift from diffusion to a chemical and therefore probably enzymatic reaction. Such observations suggest that the search for a possible primary metabolic effect of insulin should not yet be fully abandoned. These considerations led us to investigate pigeon breast muscle, where Krebs had found effects of insulin on respiration maintenance which had been confirmed. The fact that such an effect appeared to be unique with pigeon muscle seemed, essentially, not to argue against its possibly reflecting an aspect of insulin activity.

Although with the pigeon breast muscle system no insulin effect could be obtained when measuring glycogen synthesis, we continued because we became aware, during these studies, of some seemingly unresolved problems in the field of glycogen synthesis proper. Certain problematic aspects of the relation of phosphorylase to glycogen synthesis had been brought out already through work on the epinephrine effect on phosphorylase activity. This work, done mainly by Sutherland and his coworkers and by Cori, showed clearly that phosphorylase activation invariably led to glycogen breakdown and never to glycogen synthesis. Since our experiments with breast muscle homogenates gave strong support to the proposition that phosphorylase was not involved in glycogen synthesis, we considered the possibility that the synthesis might go by way of UPDG. The demonstration by Leloir that liver contains an enzyme which will form glycogen from UDPG, made it seem likely that the problem of muscle glycogen synthesis could be explained in terms of a UDPG pathway. In the experiments reported below, results with the pigeon breast muscle homogenate are described, and preliminary data are given on the rabbit and rat skeletal muscle system which synthesizes glycogen from UDPG.

Robbins, Traut and Lipmann, in "Glycogen Synthesis from Glucose, Glucose-6-Phosphate, and Uridine Diphosphate Glucose in Muscle Preparations," from *The Proceedings of the National Academy of Sciences* 45:6, 1959.

Insulin-like Effects of Serum Albumin and Globulin Fractions on Glucose Uptake by Rat Epididymal Adipose Tissue

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A method of bio-assay for insulin-like material utilizing glucose uptake by rat epididymal adipose tissue demonstrates sensitivity to as little as 10 micro-units of insulin per milliliter.[1] In this type of bio-assay, "nonspecific" insulin-like factors must be considered. This paper presents investigations of such possible activity associated with protein. Specificity of insulin-like activity is further elucidated in this paper by studies with serum protein fractions.

METHODS

The method of insulin assay, utilizing glucose uptake by rat adipose epididymal tissue, has been described in detail.[1] Concentrations of commercial human serum albumin,* varying from 0.2 per cent to 5 per cent concentration, have been tested in this system to determine the effect of protein on glucose uptake by adipose tissue. Adipose tissue segments weighing 40 to 100 mg., from rats in the 100 to 200 gm. weight range, were incubated in Krebs-bicarbonate buffer with an initial glucose content of 2.0 to 2.6 mg./beaker. Incubation proceeded for two to four hours in a Dubnoff metabolic shaker under 95 per cent O_2-5 per cent CO_2, at a temperature of 36.5°.

Human serum protein fractions were tested under the same conditions, except that heavier animals weighing 180 to 300 gm. were employed. Generally, lower glucose uptake determinations were obtained with adipose tissue from these heavier animals. The serum protein fractions were obtained from pooled normal sera by preparative electrophoresis, as described previously.[2] A continuous flow electrophoresis apparatus† was utilized and the fractions analyzed by the Durrum analytic electrophoresis method.

Adequate controls, containing no insulin and insulin in 10 to 1,000 micro-unit/ml. concentrations, were employed in each series of determinations. Only satisfactory assays have been analyzed in this study. These have been selected arbitrarily on the basis of a minimum increment of 0.100 (log 10) by the mean of insulin over the noninsulin controls for each animal. Series of determinations obtained from adipose tissue of animals not meeting this requirement were deleted.

* Normal serum albumin (human), salt poor, USP, Hyland Laboratories, Los Angeles.

† Spinco C-P Continuous Flow Electrophoresis Apparatus.

Presented in part at the Fourth International Congress of Biochemistry, in Vienna, Austria, on Sept. 1, 1958.

RESULTS

No nonspecific "insulin-like" effect of commercial human serum albumin was evident. The highest mean glucose uptake, 4.7 mg. glucose per gram adipose tissue, was obtained with no protein in the incubation medium (table 1, figure 1). The higher protein concentrations tested, 1 per cent, 2 per cent, and 5 per cent, demonstrated glucose uptakes which were, possibly, diminished to a significant extent. The mean glucose uptake values were 3.3, 3.6, and 3.1 respectively (table 1). Mean glucose uptakes of the smaller protein concentrations, 0.2 per cent and 0.5 per cent, were 4.3 and 3.9 respectively. These values did not differ significantly from the nonprotein control.

Two-tenths per cent egg albumin demonstrated approximately the same mean glucose uptake, 4.3 mg. glucose/gram adipose tissue, as 0.2 per cent commercial serum albumin.

As indicated previously, adipose tissue from heavier animals was utilized in the study of human serum fractions, with lower glucose uptakes as a consequence.[1] The only significant insulin-like effect was associated with beta globulin and adjacent fractions (table 2, figure 2). The mean glucose uptake associated with beta globulin was 4.7 mg. glucose/gram adipose tissue; 4.2 mg. was the mean glucose uptake for the fraction having a mobility between beta and gamma globulin, and 3.8 mg. was the uptake for the fraction with a mobility midway between alpha-$_2$ and beta globulin. None of the remaining fractions demonstrated significant insulin-like activity as compared with the nonprotein control. This control was composed principally of Krebs-bicarbonate buffer containing a small amount

TABLE 1

Effect of protein concentration on glucose uptake*

Protein concentration (Per cent)†	Number of determinations	Glucose uptake (mg. glucose/gm. adipose tissue) Mean‡	90 per cent confidence limits	Significance of protein mean glucose uptake compared with non-protein control mean uptake t	P
None	21	4.7	4.0-5.5		
0.2	12	4.3	3.5-5.4	.5	>.5
0.5	18	3.9	3.4-4.4	1.6	>.1,<.2
1.0	17	3.3	2.7-4.2	2.2	>.02,<.05
2.0	15	3.6	3.0-4.3	2.0	>.05,<.1
5.0	6	3.1	1.8-5.3	2.1	>.02,<.05

*Rat weight 100-240 gm., adipose tissue weight 40-100 mg., 2.0-2.6 mg. glucose/beaker.
†Human serum albumin (commercial).
‡Antilog of geometric mean.

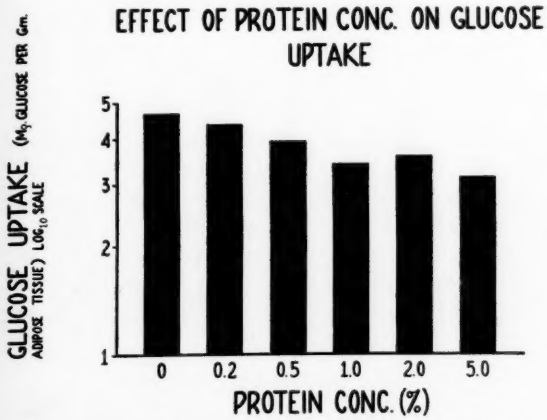

FIG. 1. Effect of varying concentrations of protein (0-5 per cent) on glucose uptake by rat adipose tissue (mg. glucose/gm. adipose tissue—log 10 graph).

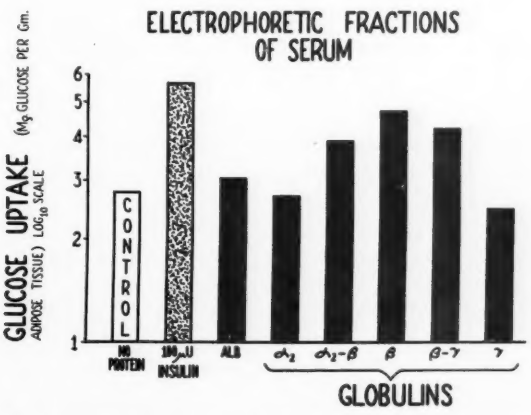

FIG. 2. Effect of serum protein fractions obtained by preparative continuous flow electrophoresis on glucose uptake by rat adipose tissue (mg. glucose/gm. adipose tissue—log 10 graph).

of the barbiturate buffer employed in the preparative electrophoretic serum protein fractionation. The mean glucose uptake of this control material was 2.8 mg./gram adipose tissue. The albumin fraction obtained concurrently by this technic of preparative electrophoresis was associated with a mean glucose uptake value of 3.0. Alpha-$_2$-globulin and gamma-globulin fractions demonstrated mean glucose uptakes of 2.7 and 2.4 respectively. Commercial human serum albumin (0.2 per cent) tested in this series of experiments had a mean glucose uptake value of 2.9. There was a significant increment of mean glucose uptake by insulin concentrations of 100 micro-units/milliliter and greater. The mean uptake for the 100 micro-unit per milliliter concentration of insulin was 5.6 mg. of glucose/gram adipose tissue.

DISCUSSION

There has been some evidence that a nonspecific stimulation of glucose uptake by rat diaphragm may be associated with plasma protein fractions.[3,4] Such a nonspecific protein stimulating effect has not been demonstrated with the adipose tissue insulin bio-assay method. There is even some statistical evidence suggesting a possible diminution of glucose uptake related to concentrations of protein greater than 0.5 per cent.

The present study of human serum fractions completely confirms the previous investigations from this laboratory utilizing the same technic of serum protein fractionation and a method of insulin bio-assay relating insulin concentration to blood glucose decrement of intact mice.[2] Both studies indicate that beta globulin and

TABLE 2

Effect of serum protein fractions on glucose uptake*

Fraction	Number of determinations	Glucose uptake (mg. glucose/gm. adipose tissue) Mean‡	90 per cent confidence limits	Significance of protein fraction mean glucose uptake compared with control mean uptake* t	P
Nonprotein control	21	2.8	2.4-3.2		
Alpha$_2$ globulin	4	2.7	2.1-3.5	0.1	>.9
Between alpha$_2$ and beta globulin	19	3.8	3.1-4.8	2.2	>.02,<.05
Beta globulin	33	4.7	4.2-5.3	4.8	<.001
Between beta and gamma globulin	31	4.2	3.6-5.0	3.0	<.01
Gamma globulin	11	2.4	1.6-3.6	0.9	>.3
0.2 per cent commercial serum albumin	25	2.9	2.5-3.3	0.5	>.6
100 μU/ml. insulin	6	5.6	4.4-7.1	3.8	<.001

*Rat weight 180-300 gm., adipose tissue weight 40-100 mg., total glucose 2.0-2.6 mg./beaker.
†Antilog of geometric mean.

adjacent serum protein fractions possess endogenous serum insulin-like activity. A recent report from the Netherlands of Bolinger and others[5] also stated that beta globulin contains the predominant endogenous serum insulin-like activity. These workers employed glucose uptake by the rat diaphragm as the insulin bio-assay method and fractionated serum proteins by starch-column electrophoresis. They also indicated that radioactive labeled insulin I^{131} was recovered in the beta-globulin fractions. Randle and Taylor[6,7] utilizing similar technics noted "bound" insulin to be associated with serum protein migrating in the beta-gamma globulin zone. They also observed that "free" insulin appeared to migrate with the slow-moving segment of albumin or with alpha$_1$-globulin. These results are consistent with the findings of Antoniades[8] and of Berson and Yalow.[9]

The difference in electrophoretic mobilities between "free" and "bound" insulin must be interpreted with caution. The "bound" insulin supposedly represents endogenous insulin. However, methods of insulin bio-assay are not specific for insulin, and substances of biological importance other than insulin may possess "insulin-like" activity, as has been previously emphasized.[2] It has been shown that an endogenous plasma insulin-like factor, unlike crystalline insulin, can be adsorbed by a cationic exchange resin from which it may be subsequently eluted.[10,11] More recently, Walaas and others[12] have described a dialyzable serum insulin-like factor of small molecular weight that is probably not insulin. Furthermore, they have noted "insulin-like" activity of certain individual amino acids at low concentrations. Leonards,[13] employing the rat epididymal fat pad for measurement of insulin-like activity, has been unable to extract the serum factor responsible for this activity with acid-alcohol or to block this activity with specific insulin anti-sera.

SUMMARY

Various concentrations of commercial human serum albumin demonstrate no nonspecific insulin-like activity as tested by a method of insulin bio-assay utilizing glucose uptake by rat epididymal adipose tissue. Serum protein fractions obtained by preparative continuous flow electrophoresis have been assayed in this system. Endogenous serum insulin-like activity appears to be associated with beta globulin and adjacent fractions containing beta globulin.

SUMMARIO IN INTERLINGUA

Effectos Insulinoide Exercite per Albumina Seral e per Fractiones de Globulina Super le Acceptation de Glucosa per Histos Adipose Epididymal del Ratto

Varie concentrationes de human albumina seral de provenientia commercial demonstrava nulle nonspecific activitate insulinoide in tests effectuate per medio de un methodo de bio-essayage de insulina basate super le studio del acceptation de glucosa per histos adipose epididymal de rattos. Fractiones de proteina seral, ob-

tenite per electrophorese a fluxo continue esseva essayate in iste systema. Il pare que le endogene activitate insulinoide in le sero es associate con globulina beta e fractiones adjacente que contine globulina beta.

ACKNOWLEDGMENT

Aided by Grant No. A-1516, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service, and by a grant from the Los Angeles Diabetes Association, Inc.

The authors wish to express their gratitude to Mrs. Rose Fishler for technical assistance, to Dr. Frederick Moore for advice concerning statistical analysis of the data, and to Dr. Helen E. Martin for her help and support.

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One of the cornerstones of contemporary biochemical thinking is the concept of the individual enzyme as the unit of enzymatic action. According to this view, every biochemical reaction is catalyzed by a specific enzyme; and all multistep processes can be accounted for by the collaboration of a group of discrete, specific enzymes. Furthermore, the characteristics of any enzyme-catalyzed sequence can be predicted on the basis of a detailed knowledge of the properties of the individual enzymes which are concerned in this process. Any number of examples appear to vindicate fully these working assumptions. The successful reconstruction of highly complex processes, such as glycolysis, fatty acid oxidation, purine synthesis, and the pentose and urea cycles, with combinations of isolated enzymes can be evaluated as impressive support for the thesis that certain multi-enzyme processes are merely the sum of the individual enzymatic reaction.

By contrast, this approach has utterly failed when applied to enzymatic processes which are intimately bound up with subcellular structure. Processes such as electron transport, oxidative phosphorylation, and photosynthesis have been found to be wedded to mitochondrial or chloroplast structure, and it has not been possible to deal with these processes by classical methods.

From an article by David E. Green and Johan Jarnefelt in *Perspectives in Biology and Medicine,* Vol. 2, page 163, 1959.

Biologists realize more keenly now than they did not more than a decade ago the crucial part played by phosphorus in the processes of life. Practically every form of energy exchanged inside the living cell is now recognized to involve the making and breaking of what are called "high energy bonds" that link oxides of phosphorus to carbon compounds or carbon nitrogen compounds. Every biological or physiological event involves gain or loss of energy, and accordingly we are entitled to infer that these "energy" rich phosphorus bonds have always played a crucial role, even from the very start of the most primitive manifestation of life.

From "Phosphorus as a Factor in the Origin of Life," by Addison Gulick, in *The American Scientist,* volume 43, page 479.

On the Mechanisms of Monosaccharide Absorption in the Small Intestine

A Review

Thomas N. Vincent, M.D., Denver

A large body of evidence has accumulated since 1900 which indicates that sugars are absorbed by more than one mechanism in the small intestine. The passage of most monosaccharides can be ascribed to diffusion phenomena; they are said to be "passively transported." There is, however, a select group of sugars and sugar-derivatives that are rapidly absorbed by the small intestine. The passage of these substances cannot be ascribed to diffusion phenomena alone, and they are said to be "actively transported." The observations that have led to the concept of active transport and have served to set it apart from passive transport are the following:*

1. There is a high structural specificity in the absorption of sugars across the intestinal wall. Slight structural modifications of a sugar may completely alter the rate of transport of the substance across the intestinal wall. Certain hexoses, for example, are transported much faster than others. All hexoses diffuse at about the same rate, so that this difference in absorption rate is not expected if the mechanism of absorption is ascribed to diffusion alone. Pentoses are known to diffuse slightly faster than hexoses. Certain hexoses, however, are absorbed much faster than pentoses and this is also not consistent with transport by diffusion alone. (Hédon, 1900; Nagano, 1902; Cori, 1925; Macleod, Magee, and Purves, 1930; Auchinachie, Macleod, and Magee, 1930; Wilbrandt and Laszt, 1933; Verzar and McDougall, 1936; Westenbrink, 1936; Csáky, 1942; Naitô, 1944; Vidal-Sivilla, 1958; and others.)

2. According to Fick's law of diffusion, the rate of transport of a substance is a linear function of the difference in concentration of the substance on both sides of the intestinal wall. Actively transported sugars do not strictly adhere to this law. Saturation phenomena occur, especially at higher concentrations. (Cori, 1925; Verzar, 1935; Vidal-Sivilla, 1950.) More recently, Fisher and Parsons (1953a, 1953b) have shown that the absorption of glucose or galactose apparently follows an equation of the Michaelis-Menten type in an in vitro rat intestine preparation. Crane and Wilson (1958) have shown a similar situation occurs with the absorption of 1-deoxy- and 6-deoxyglucose in an in vitro hamster intestine preparation.

3. Simultaneous absorption of more than one actively transported sugar leads to competition of the sugars with each other for the absorptive mechanism; the rate of absorption is diminished in contrast to the rate of absorption of individual sugars. A passively transported sugar does not diminish the rate of absorption of an actively transported sugar when the two are absorbed simultaneously. (Cori, 1926; Fisher and Parsons, 1953b; Darlington and Quastel, 1953; and Riklis, Haber, and Quastel, 1958.)

4. Active transport is dependent upon the maintenance of a rather narrow temperature range whereas passive transport is less sensitive to temperature change. (Hewitt, 1924; Auchinachie, Macleod, and Magee, 1930.)

5. Active transport is accomplished in a narrow, nearly neutral *p*H range; passive transport is less affected by *p*H changes. (Laszt, 1935a.)

6. Active transport is dependent upon a simultaneous supply of oxygen, whereas passive transport is not. (Darlington and Quastel, 1953; Wilson and Vincent, 1955; Fridhandler and Quastel, 1955.)

7. Active transport is inhibited by various enzyme poisons, the most thoroughly studied inhibitors of active transport being phlorizin and monoiodoacetic acid; passive transport is not similarly affected. (Nakazawa, 1922; Wilson, 1932; Wilbrandt and Laszt, 1933; Lundsgaard, 1933; Wertheimer, 1934; Donhoffer, 1935; Oh-

*References cited are representative of these observations. No attempt has been made to give all of the references pertinent to any one point.

Winner of the 1958-59 Medical Student-Intern Essay Contest of the American Diabetes Association for the best review article or case report. This is his prize-winning paper.

Senior Student, University of Colorado School of Medicine, Denver, Colorado. Present address: Walter Reed Army Hospital, Washington 12, D.C.

nell and Höber, 1939; Brückner, 1951; Jervis, Johnson, Sheff, and Smyth, 1956; Riklis and Quastel, 1958b.)

8. Passage of actively transported sugars may be shown to occur from a lower to a higher concentration (transport against a concentration gradient). This is opposite the thermodynamic tendency, and is the only positive proof that forces other than diffusion are involved in active transport according to Rosenberg (1954). The sugars that have been demonstrated to be actively transported by this criterion are listed in table 1.

THE PHOSPHORYLATION THEORY

At present, the phosphorylation theory is the most widely held theory that seeks to account for the phenomena of active transport. As first proposed, selectively absorbed (actively transported) sugars are phosphorylated within the intestinal epithelial cells thereby increasing the diffusion gradient of the free sugar into the cell. A refinement of the theory, proposed by Lundsgaard (1933), Kalkar (1937), and others, envisions penetration of the sugar into the cell with phosphorylation of the sugar at the luminal end of the cell, diffusion of the sugar-phosphate across the cell, and dephosphorylation at the other (basal) end of the cell with release of the free sugar. The evidence offered in support of the theory by its originators falls into three categories:

1. Wilbrandt and Laszt (1933) showed that the rate of absorption of glucose and galactose from the intestine was about the same as that of xylose, mannose, and arabinose in rats poisoned with monoiodoacetic acid whereas in normal animals, glucose and galactose are absorbed three to four times faster than the latter sugars. This difference could not be explained by the assumption of a decrease in the general permeability of the mucous membrane, for if this were the case, the passage of the passively transported sugars would also be decreased. It had been shown by Lundsgaard (1930a,b,c) that monoiodoacetic acid inhibits muscle metabolism and yeast fermentation. Yamasaki (1930) showed that yeast fermentation poisoned with monoiodoacetic acid would continue if hexosephosphate is substituted for glucose as the substrate. It was inferred from this data that monoiodoacetic acid inhibits the phosphorylation of glucose. (This later was found to be an indirect effect. Monoiodoacetic acid inhibits the energy formation processes necessary for the production of hexose-6-phosphate.) Wilbrandt and Laszt theorized that glucose and galactose are actively absorbed because they are phosphorylated; monoiodoacetic acid inhibits phosphorylation thereby inhibiting selective absorption. These results were confirmed by Lundsgaard (1933).

2. Laszt (1935b) showed that mucosal extracts of rat small intestine would phosphorylate glucose, galactose, and fructose, but not mannose or xylose. Phosphorylation was measured by the disappearance of phosphates and was inhibited by the addition of monoiodoacetic acid. This led to the conclusion that only those sugars which are actively absorbed by the intestine are phosphorylated and vice versa.

3. Laszt and Süllmann (1935) observed an accumulation of acid soluble organic phosphates (mostly hexose phosphate esters) in the intestinal mucosa of rats following the administration of glucose, galactose, and fructose. No such accumulation followed the administration of sugars not actively transported—xylose, arabinose, mannose or sorbose. This observation has been confirmed by Lundsgaard (1939) and Beck (1942).

The phosphorylation theory as proposed is useful in accounting for the phenomena of active transport: Differences in phosphorylation rate may account for the high structural specificity of the intestine. The phosphorylation theory is an enzymic mechanism which may account for the effects of saturation, competition, tem-

TABLE 1

Sugars and sugar derivatives transported against a concentration gradient

Investigators	Method	Animal	Substance tested
Bárány and Sperber (1939)	in vivo	rabbit	D-glucose
Campbell and Davson (1948)	" "	cat	3-methyl-D-glucose
Fisher and Parsons (1949)	in vitro	rat	D-glucose
Fisher and Parsons (1953b)	" "	"	D-galactose
Wilson and Wiseman (1954)	" "	hamster	D-glucose, D-galactose
Wilson and Vincent (1955)	" "	"	3-methyl-D-glucose
Crane and Krane (1956)	" "	"	1-deoxy-D-glucose
			6-deoxy-D-glucose
Wilson and Crane (1958)	" "	"	7-deoxy-D-glucoheptose
			D-glucoheptulose
			6-deoxy-D-galactose
			D-allose

perature, *p*H, dependence on simultaneous oxygen supply, and enzyme inhibitors on active absorption. Lastly, transport against a concentration gradient can be accounted for by assuming a phosphorylating mechanism.

Observations Which Complicate the Phosphorylation Theory

The phosphorylation theory, as it is usually envisaged, requires a phosphorylating enzyme at the luminal end of the epithelial cell and a dephosphorylating enzyme at the basal end of the cell. Drabkin (1948) has assumed that hexokinase is the phosphorylating enzyme and intestinal alkaline phosphatase is the dephosphorylating enzyme. Many observers have shown phosphorylating activity of mucosal extracts (presumably hexokinase activity) but the histological location of hexokinase has not yet been demonstrated. There is a large amount of alkaline phosphatase activity in intestinal mucosa, but histologically this phosphatase is located mostly in the brush border of the epithelial cells adjacent to the lumen (Gomori 1941). According to the phosphorylation theory, this is the wrong end of the cell for dephosphorylation to occur. It should be pointed out, however, that so long as (1) the spatial relationship of the two enzymes is maintained, with the phosphorylating enzyme closer to the luminal end of the cell than the dephosphorylating enzyme, and (2) the free sugar can diffuse freely through the cell, active transport may still take place regardless of the location within the cell of the dephosphorylating enzyme, since the integrity of the sugar-phosphate carrier mechanism is still maintained. Recently, Chiquoine (1955) has demonstrated the presence of a glucose-6-phosphatase at the *basal* end of the intestinal epithelial cells, and has proposed that this may be the enzyme responsible for dephosphorylation in glucose transport. Sugar phosphates other than glucose-6-phosphate presumably are formed when other sugars are actively transported, and it is not known yet whether these esters may act as substrates for this enzyme.

Althausen and Stockholm (1938) found that the rate of absorption of glucose in rats is increased after the administration of thyroxin and decreased after thyroidectomy. After ruling out various other possible causes, they suggest that thyroxin increases the intestinal absorption of glucose through stimulation of the phosphorylation mechanism. Eiler, Stockholm, and Althausen (1940) compared the accumulation of acid soluble phosphate esters in the intestinal mucosa during the absorption of glucose at accelerated and depressed rates via alteration of thyroid activity. No difference in accumulation of esters was found. The authors concluded that their data neither supported nor invalidated the phosphorylation theory since a difference in the turnover-rate of acid soluble phosphates could account for the differences in absorption.

Naitô (1944) has shown the rapid absorption of ribose as compared to other pentoses. He has also observed the accumulation of phosphate esters in the mucosa during its absorption. He concludes that ribose is phosphorylated and absorbed by an active process. Other observers, however, have not demonstrated the active absorption of ribose and have concluded that it is absorbed by a passive process (Wilson and Vincent, 1955). Hele (1953) has shown that mannose and xylose are phosphorylated by the intestinal mucosa. Thus it is apparent that some sugars are phosphorylated but not actively transported. This is not necessarily in contradiction to the phosphorylation theory if one assumes that the rate-limiting process in sugar transport is the rate of phosphorylation of the sugar, i.e., that actively transported sugars are rapidly phosphorylated, but passively transported sugars are slowly phosphorylated. Bissegger and Laszt (1951), Hele (1953), and Csáky (1953) have shown that, in general, the rate of phosphorylation of sugars by mucosal extracts in vitro parallels the rate of absorption of these sugars in vivo. Sols (1956), however, has re-evaluated the methods used for testing the phosphorylating activity of mucosal extracts and points out an inherent defect in the procedures used in the previous investigations. What was presumed to be a very brief phosphorylation of galactose and 3 methylglucose turns out to be an artifact, presumably phosphorylation of endogenous substrates in the unfasted rat intestinal mucosa. In his experiments, Sols finds a drastic lack of correlation between the rate of phosphorylation and the rate of absorption: Galactose and 3-methylglucose, both actively transported sugars, are phosphorylated at about the same rate as xylose, a passively transported sugar. On the other hand, the passively transported sugars, 2-deoxyglucose, glucosamine, and mannose, are phosphorylated nearly as rapidly as glucose.

The actively transported sugars, glucose and galactose, are glycogenic in the rat. Campbell and Davson (1948) have shown that 3-methylglucose is not glycogenic in the rat and reason that it therefore would not be expected to be actively transported. The fact that it is actively transported in the rat and cat in their experiments suggests to them that phosphorylation is not the only mechanism of active transport in the case of this sugar. They do not give direct evidence of the failure

of the intestine to phosphorylate 3-methylglucose, but Sols, as mentioned above, has shown very little phosphorylation of this sugar by rat mucosal extracts.

The phosphorylation of hexoses presumably occurs only at the 1- and 6-positions. Recently, active transport of hexoses substituted in these positions so that phosphorylation cannot take place has been demonstrated. Crane and Krane (1956) have shown that 1-deoxyglucose and 6-deoxyglucose are transported against a concentration gradient. They concluded that these data render the phosphorylation theory untenable. This conclusion is open to criticism on the basis that phosphorylation may occur in one of these positions when the other is not available because of substitution. The data will not be conclusive until the demonstration of active transport of a sugar substituted in both the 1- and 6-positions at the same time.

OTHER MECHANISMS PROPOSED FOR THE INTESTINAL ABSORPTION OF SUGARS

Earlier theories of absorption from the intestine are well reviewed by Goldschmidt (1921), Magee (1930), and Verzar and McDougall (1936). None of these explains the phenomena of active absorption.

Hestrin-Lerner and Shapiro (1953a,b, 1954) described a series of in vivo and in vitro experiments in which glucose was actively absorbed from the lumen of rat intestine but wherein they could not recover glucose from the serosal side. They suggested that glucose was absorbed as an unidentified metabolite and reconverted into glucose in some other organ, presumably in the liver. Wilson and Wiseman (1954) suggested that the unidentified metabolite might be lactate. Many investigators have since published experiments showing that most of the glucose disappearing from the lumen during absorption reappears, apparently unchanged, as glucose in the mesenteric blood. Kiyasu, Katz, and Chaikoff (1956) administered uniformly labeled C^{14}-glucose to loops of rat intestine and found that 82 to 92 per cent of the C^{14} recovered in the mesenteric blood was present as glucose, with 4 to 16 per cent present as lactate. They also analyzed the intestinal wall of the loops after absorption and found 62 to 73 per cent of the C^{14} present in the glucose fraction, with smaller amounts in lactate and alanine, and not over 9 per cent unidentified. Taylor and Langdon (1956) administered glucose-1-C^{14} by stomach tube and intraperitoneally to rats, and then determined the distribution of isotopic carbon in the glucose from the liver glycogen by degrading the glucose one carbon atom at a time. They found that the distribution of isotopic carbon was the same whether the glucose-1-C^{14} was administered by stomach tube or intraperitoneally. In both cases most of the radioactivity was found in C-1 with the activity present in the other carbon atoms distributed almost equally among them. They interpret their data as indicating that cleavage of the six-carbon chain of glucose during the process of intestinal absorption and transport to the liver is of minor quantitative significance, and state that over 90 per cent of the absorbed glucose found in the liver glycogen must have been absorbed by the intestine and transported to the liver as a six-carbon unit. Hawkins and Wills (1957) drew similar conclusions from their experiments in which they fed glucose-1-C^{14} by stomach tube to rabbits and analyzed the peripheral blood from an ear vein for radioactive substances. Glucose was isolated and degraded to a mesoxalic aldehyde containing carbons 1, 2, and 3. Most of the radioactivity was in this fraction. Atkinson, Parsons, and Smyth (1957) showed in dogs that 65 to 80 per cent of the uniformly labeled glucose-C^{14} disappearing from the intestinal lumen could be accounted for as glucose in the mesenteric blood, with the rest accounted for as lactic acid (7 to 17 per cent) and insignificant amounts as other substances. They indicated that when experimental conditions were poor, and there was significant anoxia, 30 to 40 per cent of the C^{14} was recovered as lactic acid.

Rosenberg and Wilbrandt (1952) have proposed a further refinement to the phosphorylation theory. Many investigators have observed similarities between the absorption processes of the intestinal epithelium and the reabsorption processes of the proximal kidney tubules and have assumed the mechanism is the same for both processes. On the basis of renal tubular function data and a calculated permeability figure for the cell membrane, they find a disparity between the observed rate of penetration of the cell membrane by glucose and the rate of penetration of glucose into the cell if this penetration is assumed to be on the basis of diffusion alone. To account for this they propose that sugars are phosphorylated at the cell membrane as a precondition to passage into the cell. The cell membrane is assumed to be practically impermeable to the free sugar. In other respects, the theory is unchanged.

Several other experiments have called attention to the importance of the cell membrane in the active transport of sugars. Fisher and Parsons (1953a,b), as mentioned before, find that the absorption of glucose and galactose follows Michaelis-Menten kinetics. They point out that if a substance penetrates a cell as a result of: (1) combination with or adsorption on to a component of the cell membrane, (2) passage of the

complex across the membrane, e.g., by thermal agitation, and (3) dissociation of the complex at the inner border of the cell membrane, then the kinetics of penetration would be identical with Michaelis-Menten kinetics, providing step (1) above is the rate-limiting reaction. They also point out that the Langmuir adsorption isotherm has exactly the form of the Michaelis-Menten equation, so that if adsorption of glucose on to some carrier complex were the rate-limiting process, the same kinetics would obtain. Newey, Parsons, and Smyth (1957) and Parsons, Smyth, and Taylor (1958) have concluded from recent data that the site of action of phlorizin inhibition of the absorption of glucose is the mucosal border of the cell, where it prevents glucose from entering the cell.

Keston (1954) discovered a mutarotase in renal tubular cells of the rat, and has proposed a theory of active transport of sugars based upon this enzyme. The mutarotase catalyzes the interconversion of various forms (α,β) of sugars toward an equilibrium mixture. During this interconversion a preferentially absorbed form is presumably formed which passes into the blood, where it must be present in lower concentrations according to the theory. The mutarotase, by controlling the rate of formation of a form of sugar which can penetrate the cell membrane, or a form which acts as a substrate for other enzymes, is thus a rate-limiting step in the absorption process. Keston did not, however, demonstrate an intestinal mutarotase. Moreover, Crane and Krane (1956) dispute this theory on the basis of their finding that 1-deoxyglucose is transported against a concentration gradient by hamster intestine. Substitution in the 1- position precludes the action of a mutarotase.

The absorption of fructose requires special consideration. In the many studies where comparative rates of absorption of various hexoses and pentoses have been considered, the position of fructose has always been intermediate between the passively absorbed sugars and the selectively absorbed sugars. Fructose is undoubtedly phosphorylated by the intestine during the absorption process. (Laszt and Süllmann, 1935; Lundsgaard, 1939; Beck, 1942; Kjerulf-Jensen, 1942; and Papadopolous and Roe, 1957.) Verzar and McDougall (1936) have reviewed earlier experiments and conclude that fructose is converted to glucose during the process of absorption, and that fructose which appears on the serosal side with concentrations in the lumen above 6 per cent is there as a result of diffusion. Other investigators have agreed with this view. Darlington and Quastel (1953), using guinea pig intestine in vitro, inferred from their data that *any* fructose which appears on the serosal side passes through the intestine by diffusion, since the rate of transport is the same order of magnitude as sorbose. Wilson and Vincent (1955) have shown with hamster intestine that fructose which reaches the serosal side appears to move only by passive diffusion; the glucose which is formed from fructose, however, is transported against a concentration gradient to the serosal side. Fridhandler and Quastel (1955), using guinea pig intestine, have shown that anaerobiosis or the presence of the enzyme inhibitor 2,4-dinitrophenol suppresses glucose formation from fructose but does not prevent fructose absorption. They also find that, at the concentrations used in their experiments, phlorizin has no effect on the rate of fructose absorption or on glucose formation from fructose. They state that their results conform with the conclusion that there is no "active" absorption of fructose and that glucose formation from fructose in the intestine is enzymatically controlled.

There appears to be an important species quantitative difference in the amount of glucose formation from fructose. Kiyasu and Chaikoff (1957), using C^{14}-labeled fructose, found that in isolated loops of rat intestine about 10 per cent of the absorbed fructose was recovered as glucose in the plasma that drained the loops. In the case of guinea pigs, the conversion of fructose to glucose was much higher, in the neighborhood of 70 per cent. Significant amounts of the C^{14} of absorbed C^{14}-fructose were recovered as C^{14}-lactate in the rat (up to 60 per cent in one rat). Lesser amounts of C^{14} were recovered as C^{14}-lactate in the guinea pig. These experiments are interesting in the light of Wilson's recent work (1956) on the role of lactic acid in glucose absorption. Wilson suggests that one of the mechanisms of glucose absorption from the lumen of rat intestine is the conversion of glucose to lactate in the epithelial cells followed by the preferential discharge of lactate into the blood. Presumably the liver then converts the lactate in the portal blood to glycogen. He believes that lactic acid production by rat intestinal mucosa is not an artifact owing to inadequate oxygenation, but represents true aerobic glycolysis. He points out that several experiments have shown that during the absorption of glucose, the intestines of the rat, cat, and mouse produce large amounts of lactate, whereas the intestines of the hamster, guinea pig, and rabbit produce much lesser amounts.

SUMMARY

1. Most monosaccharides pass through the intestinal wall by a process of simple diffusion.

2. The presence of an "active" transporting mechanism for a select group of sugars is well established.

3. The phosphorylation theory is useful in explaining the phenomena of active transport. The evidence in favor of the theory has been challenged by many authors, and many observations have been made which complicate or conflict with it. However, a conclusive rebuttal of the theory has not yet been made, nor have any satisfactory substitutes been proposed.

4. Phosphorylation may play an important role in absorption processes but may not be the sine qua non of active transport.

5. Glucose apparently passes through the intestinal membrane and through the portal system to the liver and on into the peripheral blood stream as an intact six-carbon unit.

6. The absorption of fructose is a special case. Some fructose reaches the serosal side of the intestine by diffusion. Most, however, is converted to glucose which is then actively transported to the serosal side. There may be species differences, however, in which a greater or lesser part of the fructose is converted to lactic acid and transported to the serosal side.

7. If the experiments of Fridhandler and Quastel (1955) and those of Atkinson, Parsons, and Smyth (1957) are valid, then it appears that the absorbing intestinal cells are permeable to fructose but not to glucose before phosphorylation occurs.

8. The alternative theories of glucose absorption proposed by Hestrin-Lerner and Shapiro (conversion to an unidentified metabolite) and Keston (preferential absorption due to conversion of the sugar by a mutarotase) have not been confirmed.

9. Cell membrane and adsorption phenomena may play an important role in active transport.

SUMMARIO IN INTERLINGUA

Concernente le Mechanismos del Absorption Monosaccharidic in le Intestino Tenue: Un Revista

1. Le majoritate del monosaccharidos passa a transverso le pariete intestinal per un processo de simple diffusion.

2. Pro un gruppo selecte de sucros, le existentia de un "active" mechanismo transportatori es ben establite.

3. Le theoria del phosphorylation es utile in le explication del phenomenos del transporto active. Le argumentos in supporto del theoria es attaccate per multe autores, e multe observationes ha essite facite que conflige con illo o rende lo plus complicate. Tamen, un rejection definitive del theoria ha non ancora essite effectuate, e nulle satisfacente substituto ha essite proponite.

4. Il es possibile que phosphorylation ha un rolo importante in le processos de absorption sin esser absolutemente indispensabile in omne forma de transporto active.

5. Il pare que glucosa passa per le membrana intestinal e via le systema portal a in le hepate e le circulation peripheric de sanguine como un unitate intacte con sex carbones.

6. Le absorption de fructosa es un caso special. Un certe quantitate de fructosa arriva al latere serosal del intestino per diffusion. Le plus grande portion de illo, del altere latere, es convertite in glucosa, e isto es transferite subsequentemente per transporto active al latere serosal. Tamen, il es possibile que differentias de specie existe, secundo le quales un plus alte o plus basse percentage del fructosa es convertite in acido lactic e transportate in iste forma al latere serosal.

7. Si le experimentos de Fridhandler e Quastel (1955) e illos de Atkinson, Parsons, e Smyth (1957) es valide, il pare que le absorbente cellulas intestinal es permeabile a fructosa sed non a glucosa ante le occurrentia del phosphorylation.

8. Le theorias alternative proponite pro explicar le absorption de glucosa per Hestrin-Lerner e Shapiro (conversion in un non-identificate metabolito) e per Keston (absorption preferential in consequentia del conversion del sucro per un mutarotase) ha remanite sin confirmation.

9. Le membranas cellular e phenomenos de adsorption ha possibilemente un importante rolo in le transporto active.

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The suggestion that the pancreas contains some principle which is active in the production of ulcers is supported by earlier experiments of L. R. Dragstedt (*Arch. Surg. 44*:338, 1942), in which he observed that animals undergoing complete loss of pancreatic juice by means of an external fistula developed ulcers in 100 per cent of the cases, whereas pancreatectomy gave almost complete immunity to ulcerogenesis. E. J. Poth and S. M. Fromm (*Gastroenterology 16*:490, 1950) observed peptic ulcer formation in dogs receiving insulin and suggested that the glucagon contamination of the insulin was responsible for the production of ulcers. However, no increase in the number of ulcers was seen following the addition of glucagon to the insulin injection. They then postulated that hypoglycemia was producing the effect. This theory received support in the report of H. S. Janowitz and B. B. Crohn (*Gastroenterology 17*:578, 1951) who observed duodenal ulcer formation associated with hyperinsulinism due to a beta cell adenoma.

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Observations on the Peripheral Metabolism of Nonesterified Fatty Acids

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As Andres, Cader and Zierler[1] pointed out in 1956, although skeletal muscle represented some 40 per cent of the total body mass, and despite our wide knowledge of the metabolic activity of excised muscle and muscle extracts, we were surprisingly ignorant of the quantitative importance of various metabolic substrates in supplying the energy requirements of muscle in fasting subjects. After measuring glucose uptake, Andres and his co-workers found that its oxidation would account for only 20 per cent of the basal oxygen consumption of the muscles, and they suggested that lipids might play a major metabolic role. This view about skeletal muscle had some support from the investigations of Bing and his associates[12] who found an appreciable uptake of nonesterified fatty acids (NEFA) by cardiac muscle.

More recently it has been shown that: (1) NEFA were found present in very high concentrations in the blood even under fasting conditions; only 1 to 2 per cent of lipids were needed to account for all the unidentified substrate,[1] (2) there was observed a relative ease of transcapillary exchange of the NEFA fraction[1] so the fatty acids were efficiently taken up[3,14] and readily oxidized by skeletal muscle,[1,4,12] and (3) NEFA were found to possess a short half-life of two to three minutes, resulting in a turnover rapid enough to furnish most of the energy needs of the tissues for lipids.[3,15]

However, in none of these studies of skeletal muscle was the amount of blood perfusing the tissue measured. Thus, the main purpose of this investigation was to evaluate the role of NEFA in the metabolism of skeletal muscle in the fasting subject, measuring not only arteriovenous differences but also blood flows, thereby permitting calculations of NEFA uptake on a quantitative basis.

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METHOD

This study was done in conjunction with other investigations being carried out at the same time.[10] Subjects fasted overnight during which time any insulin treatment was omitted. All the studies were done in a hospital ward, the patients lying comfortably in bed. Arterial and venous blood samples were withdrawn simultaneously through fine polythene catheters which had been threaded through needles into the brachial artery and into an antecubital vein carefully chosen as draining the muscle compartment of the forearm. As the blood was withdrawn over a period of one minute, blood flows were measured with a venous occlusion plethysmograph. The concentration of NEFA, in mEq./L., of the arterial and venous samples, was determined by the Van der Vies modification of the Van de Kramer method.[11] All determinations were performed in duplicate by the co-author (G.S.) and were considered valid only when the titration error of each pair of samples varied less than 5 per cent.

The percentage of uptake of NEFA by the forearm muscle was calculated as:

$$\frac{(\text{mEq./L.})_{\text{art.}} - (\text{mEq./L.})_{\text{ven.}}}{(\text{mEq./L.})_{\text{art.}}} \times 100.$$

Eleven diabetic and seven control arterial and venous concentrations were obtained from fifteen patients. The tissue NEFA uptakes were calculated from the arteriovenous difference of the NEFA concentrations and the blood flow. The units used for tissue NEFA uptake were mEq./100 ml. forearm tissue/minute.

RESULTS

1. *Arterial and venous* NEFA *concentrations of diabetic and control patients.* Figure 1. A wide range of concentrations was evident in both groups, there being no specific NEFA level differentiating the diabetic from the control patients.

2. *Uptakes of* NEFA *by skeletal muscle of the forearm in diabetics and controls.* Figure 2. Significant differences in uptakes between diabetics and controls were noted. Patients with diabetes exhibited uptakes of NEFA

ARTERIAL AND VENOUS NEFA CONCENTRATIONS OF DIABETIC AND CONTROL PATIENTS

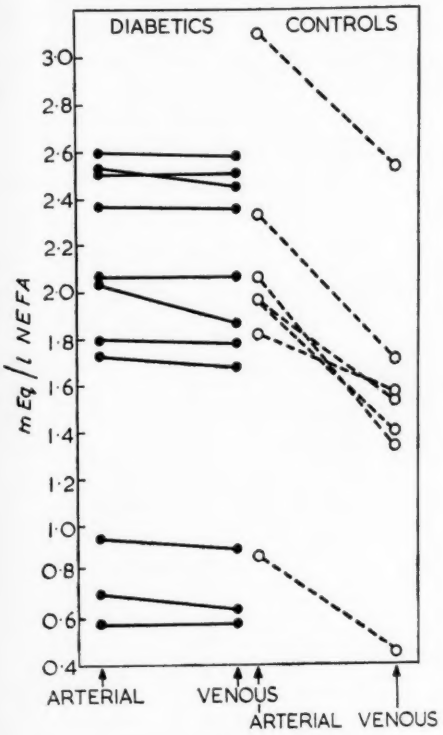

FIGURE 1

UPTAKES OF NEFA BY SKELETAL MUSCLE OF FOREARM IN DIABETIC AND CONTROL PATIENTS

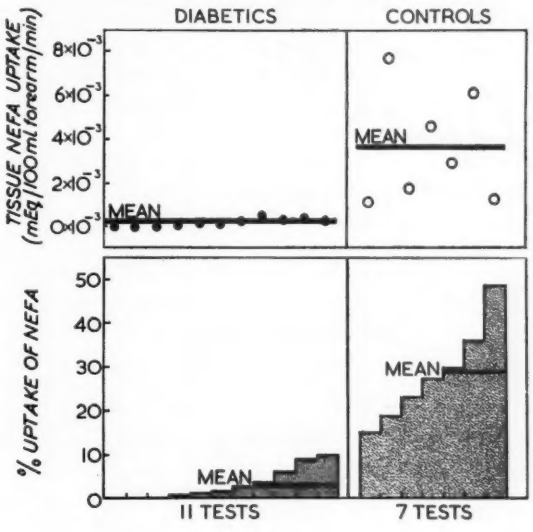

FIGURE 2

ranging from 0.0 per cent to 9.2 per cent, giving a mean of 2.9 per cent. In contrast, a much higher percentage of uptake was seen in the controls, who had a range of 14.2 per cent to 48.0 per cent, with a mean of 27.7 per cent. This mean percentage of uptake of the controls was in close agreement with the analysis of Gordon's[34] ten normal cases, in which the mean uptake was 26.9 per cent.

Correcting for blood flows, more noticeable differences were exhibited by the tissue NEFA uptakes. Diabetics showed a very minimal tissue NEFA uptake, averaging 0.17×10^{-3} mEq./100 ml. forearm/min., with a distribution of 0.00×10^{-3} to 0.44×10^{-3}. Again, the control patients' uptake was higher, ranging from 1.01×10^{-3} to 7.70×10^{-3} mEq./100 ml. forearm/min., a mean of 3.59×10^{-3}. The controls produced a wider distribution of tissue uptakes than the diabetics, who had a very limited range.

3. *Uptakes of* NEFA *by skeletal muscle of the forearm in severe and mild diabetes* (Figure 3). The role of insulin sensitivity and NEFA uptake was compared. Five diabetics were the juvenile type, whose diabetes was difficult to control and was considered relatively resistant to insulin. Three other patients (cases 3, 7, and 8) who had the mild, adult-onset type of diabetes not complicated by overweight, easily regulated and sensitive to insulin,[10] were compared to the severe cases.

The mild diabetics exhibited a higher range of percentage uptakes with a mean of 6.2 per cent as compared to 1.7 per cent uptake of the severe diabetics.

While the levels of tissue NEFA uptakes were equally distributed in both severe and mild diabetics, the latter cases had a mean tissue uptake (0.26×10^{-3}) twice that of the former (0.13×10^{-3}).

4. *The effect of insulin on* NEFA *metabolism in diabetes* (Figure 4). If the metabolic lesion of diabetes was related to inability of the forearm muscle to extract NEFA from the blood, then administration of insulin to a diabetic would be expected to correct and thereby increase the uptake of NEFA. This was the case in Patient No. 3.

Twenty-five minutes following the injection of 0.03 units of glucagon-free insulin, the diabetic's uptake increased from 0.9 per cent to 16.9 per cent, with a concomitant fall of the arterial NEFA concentration by 14.1 per cent.

5. *Repeated tests in two diabetics* (Figure 5). Over a period of more than three months, repeated tests in

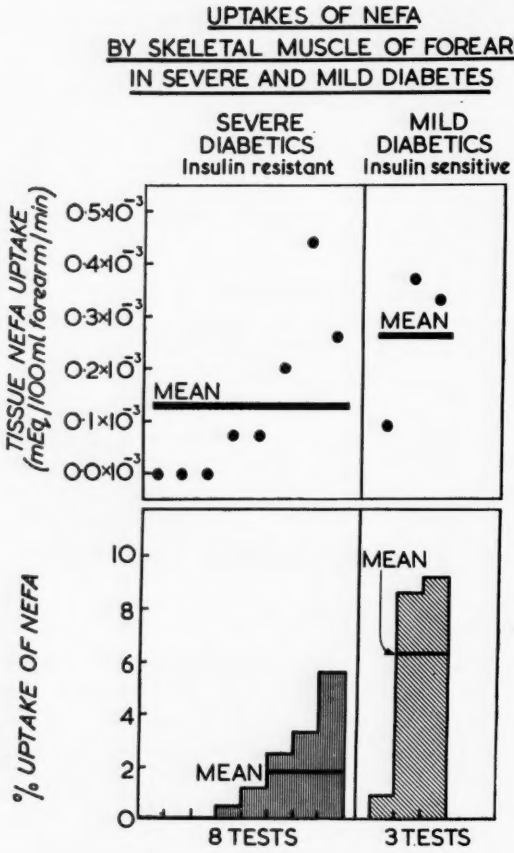

FIGURE 3

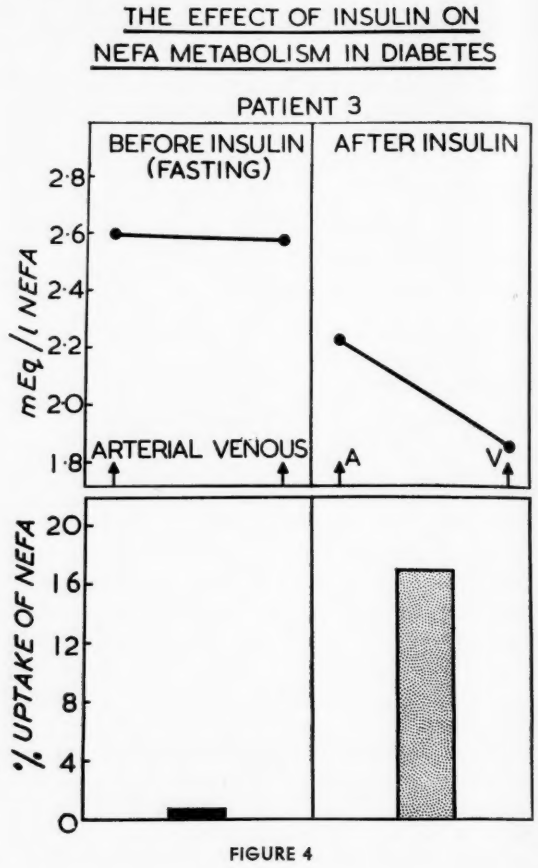

FIGURE 4

two diabetic patients showed an individual variation of NEFA concentrations. But the uptake varied less than 1½ per cent.

6. *Hepatic venous samples.* Patient No. 6, a diabetic, had an hepatic catheterization performed at the same time as the peripheral blood samples were withdrawn. The hepatic venous concentration (done in tripilicate) was 0.93 mEq./L., a value essentially the same as the peripheral venous concentration of 0.90 mEq./L.

DISCUSSION

The most likely explanation of the differences in uptakes between diabetics and controls was that normal skeletal muscle could withdraw and retain NEFA from the arterial blood for storage purposes, an ability not shared by the diabetic patients. To uphold this theory, one might cite past investigations:

1. Uptake of NEFA by skeletal muscle has been demonstrated for immediate energy needs (oxidation) or future use (storage).[5] Stadie[5] has shown that the enzymatic systems causing oxidative catabolism of fatty acids are unimpaired in diabetes. The deficiency of diabetes is the inability to synthesize fatty acids. Thus the metabolic defect in diabetes would be the anabolization of fats for storage[3,7,9] and not the catabolization of fats for oxidation.

2. The investigations of Dole[2,6] also implicate the storage depots of the body as the regulators of NEFA concentrations. In normal states of metabolism, Dole found proper utilization of carbohydrate inhibited the release of NEFA from storage depots. The diabetic patient, however, cannot control nor limit the output of NEFA from the tissue stores. Thus, Dole was of the opinion that inability to store NEFA was present in conditions of defective carbohydrate utilization, such as diabetes mellitus.

3. The diabetics' average percentage uptake of NEFA in this study probably afforded sufficient substrate for

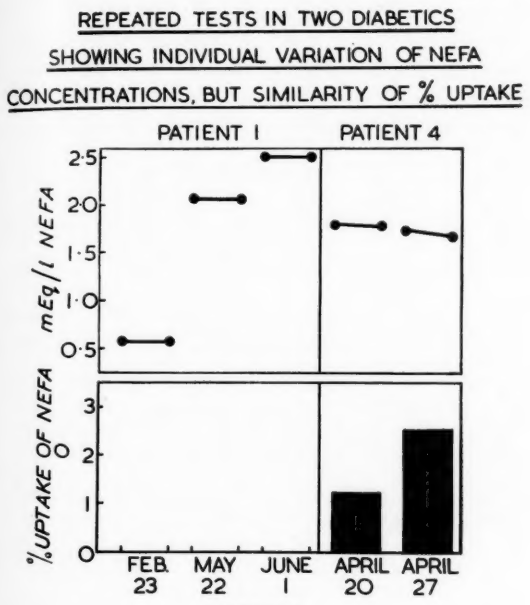

FIGURE 5

oxidation. Only 1 to 2 per cent of circulating NEFA was found necessary for immediate oxidation,[1] and the mean amount extracted by the diabetics here was 2.9 per cent.

Thus, our findings confirmed the previous investigations in which the differences of uptakes between diabetics and controls could not be explained by a deficiency of oxidation, but rather by a defect in the ability to take up and store NEFA.

In this study, other findings were of importance. First, as one progressed up the scale of insulin sensitivity, from severe diabetic to mild diabetic to control patients, an increase of the uptakes was seen. Whether this was related specifically to insulin sensitivity or to the ability to utilize carbohydrate due to other influences is undetermined. Dole[2] thought that insulin was not the sole regulator of NEFA metabolism. Second, even if other factors besides insulin govern NEFA, insulin is still an important influence. The effect of insulin on NEFA metabolism has been shown elsewhere,[2,3,6] as well as by this investigation.

TABLE 1

Case number	Initials, age, sex	NEFA (mEq./L.) Arterial	Venous	Per cent of uptake of NEFA	Blood flow	Tissue NEFA uptake units × 10^{-3}
Diabetics						
1a	DS, 36 M	0.58	0.58	0.0	5.5	0.00
1b		2.07	2.07	0.0	5.8	0.00
1c		2.51	2.51	0.0	3.4	0.00
2	JH, 47 M	2.37	2.36	0.5	7.4	0.07
3	RD, 64 F	2.60	2.58	0.9	4.6	0.09
4a	MC, 30 F	1.80	1.78	1.2	3.5	0.07
4b		1.73	1.68	2.5	4.0	0.20
5	HW, 22 M	2.53	2.45	3.3	5.5	0.44
6	JT, 20 F	0.95	0.90	5.8	5.2	0.26
7	EH, 70 M	2.04	1.87	8.6	2.2	0.37
8	FP, 64 M acromegaly	0.71	0.64	9.2	4.7	0.33
Mean				2.9	4.7	0.17
Controls						
9	AT, 43 F obesity	1.82	1.56	14.2	3.9	1.01
10	GE, 20 F hyperthyroidism	3.10	2.53	18.1	13.5	7.70
11	LB, 26 F acromegaly	1.97	1.53	22.4	3.9	1.72
12	PS, 63 M	2.33	1.71	26.7	7.4	4.59
13	JC, 24 M	1.97	1.40	29.0	5.0	2.85
14	WL, 38 M acromegaly	2.06	1.34	35.2	8.4	6.05
15	KF, 35 M	0.87	0.45	48.0	2.8	1.18
Mean				27.7	6.4	3.59

SUMMARY

Using a technic for the quantitative study of the metabolism of peripheral tissue, we have investigated the uptakes of nonesterified fatty acids (NEFA) in seven control and eight diabetic patients.

The results showed that the arterial concentrations of NEFA were the same in the two groups. However, the mean tissue NEFA uptake in the controls was twenty times more than the diabetics.

The high uptakes of NEFA in the controls suggest these fractions of fat play an important role in the peripheral metabolism of normal skeletal muscle.

Also, the mild, adult diabetics exhibited a higher uptake than the severe, juvenile diabetic patients. Administration of insulin to the diabetic patient increased the uptake of NEFA from 1 per cent to 17 per cent.

It would therefore appear that the metabolic lesion in diabetes also includes failure of the peripheral tissues to take up NEFA, a fault which may be corrected by insulin.

SUMMARIO IN INTERLINGUA

Observationes Relative al Metabolismo Peripheric de Nonesterificate Acidos Grasse

Utilisante un technica pro le studio quantitative del metabolismo de histos peripheric, nos ha investigate le acceptation de nonesterificate acidos grasse in septe subjectos de controlo e in octo diabeticos.

Le resultatos monstrava que le concentrationes arterial de nonesterificate acidos grasse esseva le mesmes in le duo gruppos. Tamen, le acceptation medie de nonesterificate acidos grasse per le histos esseva vinti vices plus grande in le subjectos de controlo que in le diabeticos.

Le alte valores pro le acceptation de nonesterificate acidos grasse que esseva observate in le subjectos de controlo pare indicar que iste fractiones de grassia ha un rolo importante in le metabolismo peripheric de normal musculo skeletal.

In plus, diabeticos adulte con leve formas del morbo exhibiva plus alte valores de acceptation que diabeticos juvenil con formas sever del morbo. Le administration de insulina al diabeticos resultava in un augmento del acceptation de nonesterificate acidos grasse ab 1 pro cento usque a 17 pro cento.

Per consequente, il pare que le lesion metabolic de diabete include etiam le non-acceptation de nonesterificate acidos grasse in histos peripheric e que iste defecto pote esser corrigite per insulina.

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Metabolism of D-Ribose in Diabetes Mellitus

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D-ribose, administered intravenously to normal subjects, disappears rapidly from blood, is sparingly excreted in urine, and produces a significant decrease in blood glucose levels.[1-4] C^{14}-labeled ribose has been traced to glucose[2,5] and CO_2[2] in man, presumably via metabolism over pentose phosphate pathways.[2,5] In addition, this sugar is an efficient glycogen precursor in several mammals.[6-8]

Ribose metabolism may be insulin dependent, since administration of this hormone enhances the removal of ribose from the blood stream.[2] On the other hand, insulin appears to have no effect on transport of this pentose into muscle cells.[9]

The hypoglycemic effect of ribose in normal subjects is a property shared by galactose,[10] but not by other pentoses[11] or fructose.[12] It is noteworthy in this regard that, of several monosaccharides tested, only glucose, galactose, and ribose stimulated the release of insulin from dog pancreas.[13] Ribose may possibly produce a diminution of hepatic glucose outflow. It has been suggested that ribose impairs hepatic glycogenolysis, since this pentose can inhibit phosphoglucomutase in vitro.[8]

Segal and his co-workers observed decreased blood glucose levels following the intravenous administration of 20 gm. of ribose to three diabetic subjects.[1] Results in the present study, using larger doses of ribose given to patients with both mild and severe diabetes, confirm this finding and show in addition that several aspects of the metabolism of this pentose remain unaltered in the diabetic state.

METHODS

Five per cent solutions of D-ribose, obtained from Pfanstiehl Laboratories, Inc., Waukegan, Illinois, were prepared using sterile pyrogen-free water and were checked bacteriologically for sterility. Four mild adult diabetic and four severe juvenile-type diabetic patients were each given an intravenous infusion of ribose (40 or 50 gm.) administered at a constant rate over a one-hour period following an overnight fast. Insulin was withheld for at least twenty-four hours before the test. Heparinized venous blood samples and timed urine collections were obtained prior to the infusion and at hourly intervals thereafter.

Blood glucose was measured directly in blood filtrates prepared by the Somogyi method[14] using the prepared enzymatic reagent, Glucostat, Worthington Biochemical Corporation (glucose oxidase, horse-radish peroxidases, phosphate buffer, and O-dianisidine) as described by Saifer and Gerstenfeld.[15] Ribose added to glucose solutions had no influence on color development by this method.

Ribose in blood was determined by the orcinol method following treatment of the filtrates with glucose oxidase (Pfanstiehl).[11] Nonesterified fatty acids (NEFA) in plasma were measured by the method of Dole.[16]

Glucose and ribose were determined on diluted urine by the methods described for blood. However, prior to incubation with Glucostat, diluted urine samples were treated with ion exchange resins to remove interfering substances. A method described by Salomon and Johnson[17] was modified as follows: Urine was diluted to contain glucose concentrations from .015 to .075 mg./ml., a range in which the color response with the glucose oxidase reagent is linear. For this purpose, preliminary estimations of urine glucose content with Tes-Tape (urine sugar analysis paper, Lilly) were found to be satisfactory. To approximately 1 gm. of a mixture of equal parts of freshly regenerated Amberlite IR 120 and Amberlite IR 45 ion exchange resins in a 13 × 100 mm. test tube, 2 to 3 ml. of the dilute urine sample were added following washing of the resin with several aliquots of the sample. The tubes were then mechanically shaken for ten minutes and the resin allowed to settle. When low urine dilutions (less than 1:25) were used, an increase in resin treatment time was found to be necessary to remove effectively all interfering sub-

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stances. Duplicate 1 ml. aliquots of resin treated dilute urine samples were then removed for incubation with the glucose oxidase reagent. By this procedure, recovery of glucose added to varying dilutions of urine was found to be essentially complete.

Urine ketopentoses were measured by a method previously described.[28] Ketones in urine were estimated with Acetone Test Reagent, Denco (sodium nitroprusside, sodium bicarbonate, and ammonium sulfate).

RESULTS

The disappearance of ribose from blood (half time = 27 ± 3 minutes) * in eight diabetic subjects was not significantly different ($p>.100$) from that observed previously in normals (half time = 23 ± 4 minutes)[4] (figure 1). Further, mild and severe diabetics removed ribose at similar rates ($p>.050$).

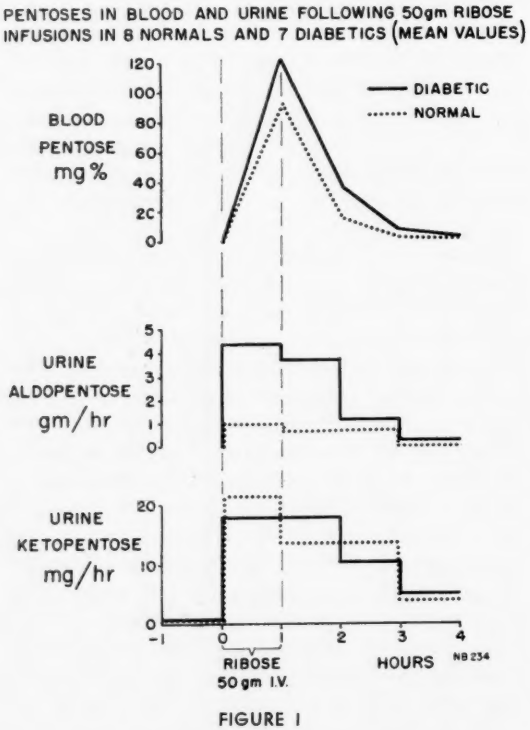

FIGURE 1

The rate of excretion of ribose in urine was greater ($p<.005$) in diabetics in the first three hours following infusion than in normals[4] (figure 1) and resulted in the elimination of 20 ± 10 per cent of the dose in four hours. Ketopentose excretion rates, however, were similar.

Blood glucose concentration decreased 21 ± 11 per cent from fasting values in seven of the eight subjects (table 1). No hypoglycemic response was observed in one diabetic (No. 8) who had been difficult to regulate. Minimum glucose values were reached one to three hours following the start of infusion. This effect was of similar magnitude ($p>.200$) in mild and severe diabetics and was not associated with symptoms. In addition, a prolonged fall in the renal excretion rate of glucose was observed following infusion (table 2).

A decrease of 33 ± 18 per cent in plasma nonesterified fatty acid concentration occurred in those subjects who responded with a fall in blood glucose (table 1). Four of these exhibited varying degrees of ketonuria prior to infusion. In all cases, urine ketones were considerably reduced in two hours (figure 2).

In one mild diabetic examined, no ribose was present in spinal fluid obtained immediately after infusion.

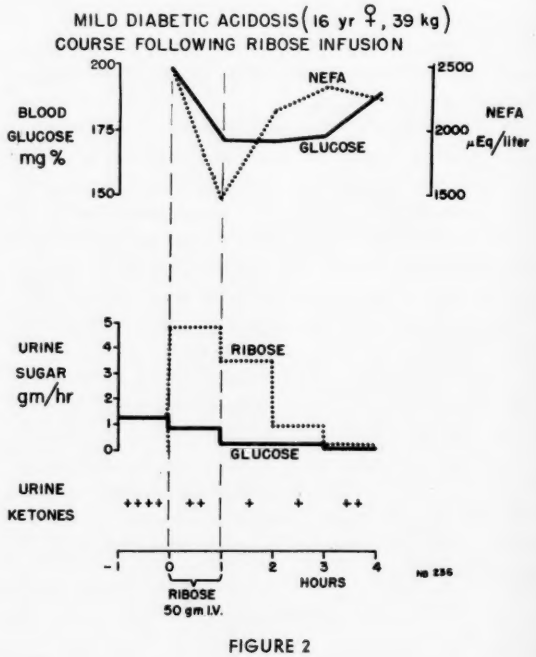

FIGURE 2

DISCUSSION

The rate of disappearance of ribose from blood was not impaired in diabetics, confirming similar observations by Segal and Foley.[3] However, results in the present study differ in that diabetic subjects excreted more than twice as much of the administered dose as has

*Data expressed as: mean for each series of observations ± the standard deviation of the individual observations.

TABLE 1

Effect of ribose* on blood glucose and nonesterified fatty acids

Subject	Sex	Age	Weight (kg.)	Insulin dose (units/day)	GLUCOSE Initial value (mg. per cent)	Per cent change Hours 1	2	3	4	NEFA Initial value (μEq./L.)	Per cent change Hours 1	2	3	4
(A) Mild diabetic														
1	M	42	79	0	87	—1	—45	—46	—33	723	—16	—20	—26	—11
2	M	46	92	35	140	13	—11	—16	—1	896	—5	+29	+56	+77
3	M	47	96	15	149	0	—6	—13	—13	1,528	—18	—25	+2	+9
4	M	48	74	0	162	—9	—19	—17	—19	742	—31	—47	—33	—9
(B) Severe diabetic														
5	F	16	39	60	200	—15	—15	—14	—6	2,481	—40	—13	—6	—9
6	F	36	50	80	188	—20	—14	—5	—13	896	—4	—25	—11	+16
7	M	17	78	100	226	—9	—17	—12	——	1,312	—44	—60	+14	——
8	M	30	80	60	129	+5	0	+9	+9	824	+4	+4	+13	+17

*Dose = 50 gm. in all cases except Subject No. 5, 40 gm.

TABLE 2

Effect of ribose on urine glucose excretion (gm./hr.)

Subject	Period following infusions (hours) Control	0 to 1	1 to 2	2 to 3	3 to 4
(A) Mild diabetic					
1	.06	.03	.01	.01	.01
2	.20	.12	.04	.03	.04
3	.06	.01	.02	—	—
4	.01	.02	.01	.01	.01
(B) Severe diabetic					
5	1.22	.83	.20	.22	.06
6	.18	.22	.03	.17	.16
7	1.14	1.24	.17	.06	.06

been reported for normals.[2-4] This suggests some impairment of ribose utilization in the diabetic, but it is possible that renal tubular reabsorption of ribose was partially blocked by glucose, a phenomenon well defined for another pentose, xylose.[19]

Although conversion of ribose to glucose occurs in both diabetic[3] and normal subjects,[5] ribose produces a decrease in blood glucose levels. This differs from the effect produced by the administration of insulin independent hexoses such as fructose and galactose, since they contribute further to diabetic hyperglycemia.[20,21] However, these experiments do not rule out enhanced conversion of ribose to glucose in diabetes. Possibly, stimulation of endogenous insulin release could account for some effect on blood glucose, since no hypoglycemia results from ribose infusions given to pancreatectomized dogs.[22,23]

As has also been noted in normal subjects,[3,4] no symptoms were observed that could be associated with the lowering of blood sugar. It is unlikely that D-ribose can be metabolized by the brain in vivo, since evidence obtained in the rat[9] and in this study indicates that the sugar does not pass the blood brain barrier.

The utilization of ribose appears to alleviate the excessive fat mobilization characteristic of the uncontrolled diabetic. The decrease in plasma nonesterified fatty acid concentration is similar to that observed when ribose is administered to normal subjects.[4] In accord with this observation, all diabetics exhibited clearing of ketonuria when present prior to infusion. This is in harmony with the demonstration that stimulation of metabolism via the pentose phosphate pathway leads to a very marked acceleration of lipid synthesis, both in normal[24] and diabetic[25] rat liver homogenates.

Several ketopentoses, intermediates in pathways of pentose metabolism, are normally excreted in urine at an average rate of 4 mg./day, predominantly as L-xylulose.[18] Dietary supplementation with carbohydrate or protein, or administration of triiodothyronine, increases urinary L-xylulose excretion, possibly reflecting accelerated metabolism via the glucuronic acid pathway.[18] A marked increase in ketopentose excretion, predominantly D-xylulose, results from the administration of ribose to normal subjects.[4] The similar high rates of excretion noted in diabetics following ribose administration suggests that insulin is not required for metabolism of ribose over pentose pathways.

Thus, a comparison of ribose disappearance rate, blood glucose levels, plasma nonesterified fatty acid concentration, and ketopentose excretion, in normal and diabetic subjects suggests that the utilization of ribose is not altered in diabetes. The increased urinary excretion of ribose in the diabetic appears contradictory.

Further study of the renal tubular reabsorptive mechanism for ribose is needed, however, before it can be concluded that this effect reflects impaired metabolism. It has been reported that, in one diabetic examined, the elimination of a tracer dose of C^{14}-labeled ribose as $C^{14}O_2$ was diminished;[8] this finding, however, has been ascribed to defective metabolism following conversion of ribose to glucose.

Since ribose can be utilized by the diabetic, does not contribute to hyperglycemia, and limits fat mobilization, further investigation of the possible beneficial effect of administration of this sugar in uncontrolled diabetes is warranted.

SUMMARY

D-ribose, given intravenously to mild and severe diabetic patients, produced a decrease in blood glucose concentration and urine glucose excretion.

Ribose disappeared from blood at a normal rate; however, increased amounts were excreted in urine. Ketopentose excretion was not altered in diabetics.

A drop in plasma nonesterified fatty acid levels paralleled the fall in blood glucose and was associated with a reduction in urine ketones.

SUMMARIO IN INTERLINGUA

Le Metabolismo de d-Ribosa in Diabete Mellite

Le administration intravenose de d-ribosa a patientes con grados leve e sever de diabete produceva un reduction del concentration de glucosa in le sanguine e del excretion de glucosa in le urina.

Le ribosa dispareva ab le sanguine con un rapiditate normal. Tamen, augmentate quantitates de illo esseva excernite in le urina. Le excretion de cetopentosa non esseva alterate in le patientes diabetic.

Un reduction del nivellos de nonesterificate acidos grasse in le plasma occurreva parallel al reduction del glucosa in le sanguine e esseva associate con un reduction del cetones in le urina.

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A Re-evaluation of in Vitro Methods for Insulin Bio-assay

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Most of the information about the concentration of insulin in plasma is based upon bio-assays performed with in vitro systems. Herein, the unknown sample of plasma is incubated with an insulin-responsive tissue and the resultant change in glucose metabolism is measured. A value for the insulin-content of the plasma is assigned on the basis of the dose-response relationships which obtain in the assay system when simple saline incubation media are supplemented with known amounts of insulin.

In the light of recent developments, these methods require re-examination. First, it has been shown in many laboratories that proteolytic mechanisms for insulin degradation are present in many tissues, and that the hypoglycemic effectiveness of insulin is destroyed by proteolysis.[1-8] Such proteolysis occurring in the conventional bio-assay systems might introduce error into the observed dose-response relationships. Secondly, work in this laboratory has demonstrated that normal fasting plasma contains protein factors which may act as non-competitive inhibitors of insulin degradation.[9] If plasma were to modify insulin proteolysis during bio-assay, the validity of comparing unknown plasma samples with known aqueous standards might be subject to error.

The present studies were initiated to assess these possibilities in the four most widely quoted in vitro systems for the bio-assay of insulin—i.e., the rat diaphragm methods of Randle,[10,11] Groen and Willebrands[12,13] and and Vallance-Owen and Hurlock,[14] and the rat adipose tissue method of Martin, Renold and Dagenais,[15] in which the epididymal fat pad is employed as the insulin-responsive tissue. The salient details of these four bio-assay procedures are summarized in figure 1.

MATERIALS AND METHODS

Tissues were prepared exactly as outlined in the individual assay procedures.[10-15] Thus, for studies with the Randle system, six hemidiaphragms were "washed for exactly five minutes at room temperature in buffer-glucose (glucose concentration 2.5 mg. per ml.),"[10] and subsequently incubated in a 1 ml. volume of the Gey and Gey[16] balanced salt solution. The Groen and Vallance-Owen systems were reproduced by immersing the four and the one hemidiaphragms which are used in these respective procedures in glucose-free, chilled, balanced saline preparatory to incubation in 2 ml. volumes of Gey and Gey media. For all of the rat diaphragm studies, male animals weighing 100 to 130 gm. were fasted for eighteen and twenty-four hours. Wet weights of single hemidiaphragms from these animals averaged 106.1 ± 23.1* mg. in eleven estimations.

	RANDLE	GROEN	VALLANCE-OWEN	MARTIN, et al
Tissue	HD (6)	HD (4)	HD (1)	FAT PAD
Medium	G & G	G & G	G & G	KRB
Volume (mL.)	1	2	2	2
Gas (O_2 % : CO_2 %)	93 : 7	93 : 7	95 : 5	95 : 5
Incubation (min.)	180	90	90	120
Plasma (mL.)	0.25	0.3	1.9	1.9
Normal Human Plasma (milliunits/mL.) *FED **FASTING	9 - 22*	0.1 - 3.0**	.031 - .082**	.05 - .35**

HD = Hemidiaphragm G & G = Gey & Gey KRB = Krebs-Ringer-Bicarbonate

FIG. 1. Systems for the in vitro bio-assay of insulin.

Epididymal fat pads were obtained from fed animals weighing 190 to 220 gm. Portions of adipose tissue were cut directly into 2 ml. volumes of Krebs-Ringer-bicarbonate medium contained within tared vessels. Filled flasks were reweighed to assess the weight of the fat pads by difference. In some instances, weights were estimated directly on a torsion balance.

Rats were Sprague-Dawley descendants secured from the Charles River Breeding Laboratories, Brookline, Massachusetts, and maintained in this laboratory on

*Mean ± Standard Deviation.

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From the Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts, and the Howard Hughes Medical Institute.

Purina pellets. Tissues were obtained within three minutes following sacrifice of the animals by a blow on the head and exsanguination.

Incubation was performed with a Dubnoff metabolic agitator (shaking rate: ninety-two cycles per minute). Suspending media were equilibrated at 38° C. and timed incubation at this temperature was initiated by the introduction of the tissues into the reaction vessels. The details of incubation intrinsic to the individual assay procedures were uniformly modified as follows:

1. All media were supplemented to a standard glucose content of 3 mg./ml. (except where indicated in the "Results" section).

2. A mixture of 95 per cent oxygen—5 per cent carbon dioxide was used as the gas phase for all systems.

3. Tracer quantities of I^{131}-insulin (v.i.) were used to document the metabolic fate of the insulin in the individual assay procedures.

4. All suspending media were supplemented with 6 mg./ml. human serum albumin (HSA) to reduce adsorption to glassware[17] and spontaneous degradation of dilute insulin solutions. Control studies have demonstrated that these methodological artifacts are more effectively eliminated by HSA than by the 100 mg. per cent gelatin that has been recommended.[15,18] The 6 mg./ml. HSA does not modify insulin degradation.

I^{131}-insulin for the present studies was obtained from Abbott Laboratories, Oak Ridge, Tennessee. Upon receipt in this laboratory, the labeled insulin was diluted with phosphate-buffered mixtures of HSA and saline (*p*H 7.4) and dialyzed as outlined elsewhere.[19] As judged by chromatographic, zone electrophoretic, and immunologic criteria, the labeled insulin retained molecular homogeneity. No preparation was employed for more than ten days following receipt in this laboratory. Under these conditions, measurable reduction in the hypoglycemic potency of the I^{131}-insulin could not be demonstrated.[19] Labeled insulin was adjusted to the desired specific activity for individual experiments with crystalline "glucagon-free" insulin provided by Dr. O. K. Behrens, Lilly Research Laboratories, Indianapolis, Indiana.

Degradation of insulin during incubation for ten and ninety minutes in the various systems was assessed on the basis of the changes in the solubility characteristics of the radioactivity in trichloracetic acid (TCA). Abundant work has indicated that this constitutes a valid index of the proteolysis and biological inactivation of insulin in vitro.[2-5,8,19] In all experiments, the tissues were removed at the end of incubation and suspending media were quantitatively decanted into 13 × 100 mm. glass test tubes containing 2 ml. of chilled 10 per cent TCA. The radioactivity in the media was partitioned into TCA-soluble and TCA-insoluble fractions by centrifugation. The insoluble residues were resuspended for washing with 2 ml. of 10 per cent TCA and repeat centrifugation was performed. TCA-supernatant fractions were pooled in 13 × 100 mm. tubes and the washed sediments were dissolved in a comparable volume of 30 per cent KOH. Control media which had been incubated without tissues were processed similarly. Diaphragms were briefly blotted and boiled in 1 ml. of water for thirty seconds. The water and boiled diaphragms were transferred to Potter-Elvehjem glass homogenizers for homogenization with 3 ml. of 10 per cent TCA. Thereafter, the contents of the homogenizers were quantitatively poured into 13 × 100 mm. tubes and fractionated into TCA-soluble and TCA-insoluble moieties as above. Separate processing of tissues and media was instituted with the diaphragm to minimize variable coprecipitation artifact. Moreover, because of the different amounts of tissue and medium in the three diaphragm procedures, total insulin degradation was assessed by combining the results of these analyses. Thus, after appropriate correction for control vessels, the sum of the TCA-soluble (nonprecipitable) I^{131} in the media and diaphragms was expressed as a percentage of the total I^{131} which was recovered from both sources. On the other hand, in the epididymal fat pad system, the contribution of tissue radioactivity was small and relatively constant. In twenty-two observations, with tissue weights ranging from 85 to 679 mg., the fat pad contained only 1.98 ± 0.98* per cent of the total radioactivity. Regardless of its nature, this amount of radioactivity could not appreciably influence estimates of insulin degradation. Thus, breakdown of insulin in the epididymal fat pad system was assessed solely on the basis of the changes in the TCA-solubility of the radioactivity within the media.

Radioactive assay of the TCA-soluble and TCA-insoluble fractions was performed by placing the 13 × 100 mm. tubes directly in a Nancy Wood well-type scintillation counter with a sensitivity of 1.0×10^6 cpm per uc. I^{131} and a background of 125 cpm. Sufficient counts were observed to reduce the probable error of the measurements to less than ±3 per cent.

Tests for the significance of differences between means and correlation coefficients were based upon the appropriate modifications of the "t" test of "Student."

RESULTS

1. Degradation of insulin by diaphragm systems in media containing 0.1 milliunit of insulin per ml.: Results are illustrated in figure 2. Values for the percentage

of the total insulin degraded in the Vallance-Owen, Groen, and Randle procedures during ten minutes of incubation were 5.5 ± 4.0* per cent, 14.8 ± 6.0* per cent, and 24.2 ± 8.3* per cent respectively. During ninety minutes of incubation, the respective values were 43.3 ± 16.3* per cent, 70.2 ± 7.5* per cent, and 72.9 ± 7.2* per cent. The differences between the three methods were statistically significant for the ten-minute interval ($p<0.05$). At ninety minutes, the difference between insulin degradation in the Groen and Randle systems was not significant ($p>0.1$);† however, the Vallance-Owen procedure still differed significantly from the other two methods ($p<0.01$). Thus, the proportional relationship between tissue mass (i.e., the number of suspended hemidiaphragms/ml. of medium) and percentile insulin degradation was most clearly demonstrated at the ten-minute interval (figure 3). At this time, insulin degradation was still relatively linear and asymptotic values had not yet been achieved.

To assess whether insulin degradation in the rat diaphragm systems is conditioned by extracellular events, the experiments pictured on the right side of figure 3 were performed. Hemidiaphragms were incubated without insulin as per Vallance-Owen, Groen, and Randle. Two milliliters of medium and twelve hemidiaphragms were employed for the Randle procedure. After ninety minutes of incubation, 1 ml. of medium was removed from the diaphragm vessels and combined with 1 ml. of fresh, insulin-containing medium to yield 2 ml. cell-free systems containing 0.1 milliunit insulin per ml. The contribution of the diaphragms to these systems was expressed in terms of "hemidiaphragm equivalents"—i.e., the number of diaphragms which had been leached per milliliter of final reaction mixture. Insulin degradation occurred in the cell-free preparations and was proportional to the number of diaphragms which had been leached (figure 3, right). In paired comparisons, during ten and ninety minutes of exposure to insulin, the systems containing the leached extracts exhibited greater insulin-degrading capacities than their counterparts containing intact hemidiaphragms (figure 3). While these studies were in progress, Narahara and Williams reported a similar leaching phenomenon.[20]

2. Degradation of insulin by rat epididymal fat pad in media containing 0.1 milliunit of insulin per ml.: Correlation of insulin degradation with tissue mass is

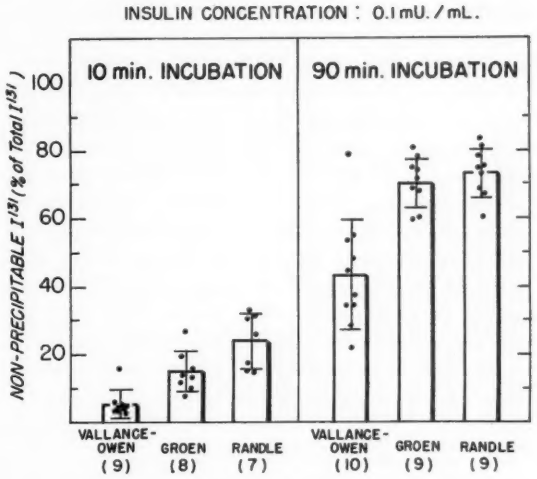

FIG. 2. The degradation of insulin in rat diaphragm bio-assay systems: Mean values for the percentage of total insulin that was degraded are indicated by the heights of the bar graphs and standard deviations by the joined transverse lines. Each point denotes an individual experiment; the bracketed numbers indicate the total number of experiments that were performed.

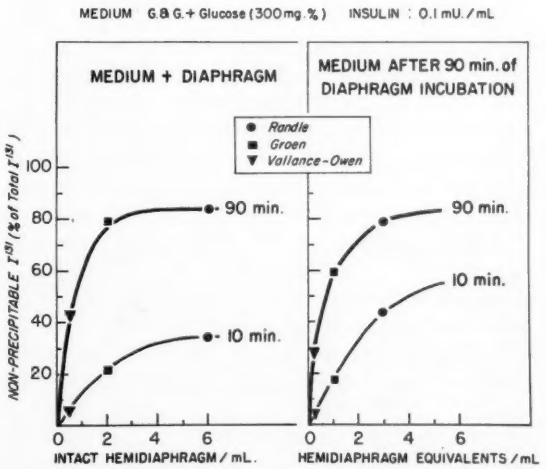

FIG. 3. Release of insulin degrading activity into the medium during incubation of rat diaphragms: Results obtained during the standard ten- and ninety-minute incubation of rat hemidiaphragms with insulin are shown on the left. Insulin degradation during ten- and ninety-minute incubation of cell-free systems with insulin is illustrated on the right. Cell-free systems had been prepared by leaching varying quantities of rat hemidiaphragms for ninety minutes. "Hemidiaphragms equivalents" refers to the number of hemidiaphragms which had been leached per milliliter of final reaction mixture. For details, see text.

†Proteolysis of I^{131}-insulin at low concentrations of insulin is not a linear function in any insulin-degrading system and asymptotic values exceeding 70-85 per cent have not been reported.[2-8] Thus, the ninety-minute values in Groen and Randle systems represent essentially complete insulin degradation.

illustrated in the thirteen experiments pictured in figure 4. In each of these studies, three differently weighing portions of adipose tissue from a single rat were incubated separately. Results were similar in both Gey and Gey and Krebs-Ringer-bicarbonate media (figure 4). For any single animal, insulin degradation was proportional to the weight of the incubated tissue. When equally weighing tissues from different animals were compared, considerable variability in insulin degradation was observed. Nonetheless, pooled values from forty-nine observations with tissues from twenty animals yielded a correlation coefficient of 0.650 ($p<0.01$) between tissue mass and insulin degradation during ninety minutes of incubation. Thus, the results obtained with the Martin, Renold and Dagenais procedure in which 80-200 mg. of tissue are recommended have been subdivided on the basis of weight:

During ninety minutes of incubation, 13.9 ± 7.9* per cent of the total radioactivity was rendered TCA-soluble in fifteen observations with 60-139 mg. pieces of adipose tissue. Degradation values during comparable intervals with 140 to 219 mg. pieces of fat pad were 19.2 ± 6.8* per cent in ten observations. The difference between the two weight groups was significant ($p<0.01$). In nineteen experiments conducted with tissues in the 80-200 mg. weight range recommended by Martin, insulin degradation at ninety minutes averaged 14.6 ± 7.9* per cent. This value was significantly less than that observed with any of the rat diaphragm methods ($p<0.01$). Values for the ninety-minute interval have been presented because of the essentially negligible insulin degradation by adipose tissue during ten minutes of incubation (table 1).

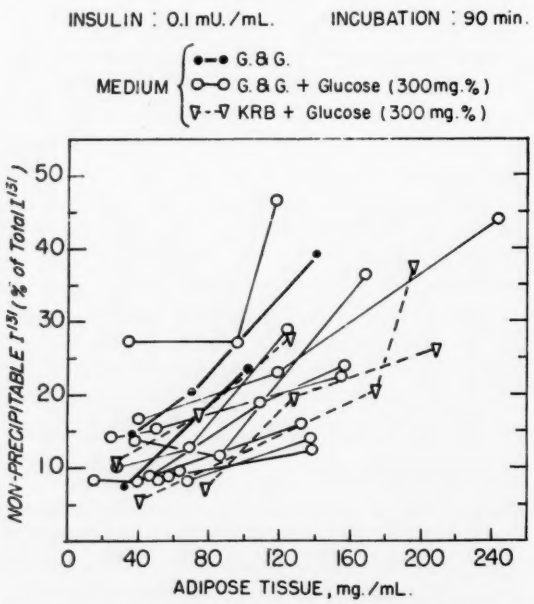

FIG. 4. Insulin degradation in the rat epididymal fat pad system: Final reaction mixture contained 2 ml. of the suspending medium denoted by the open and closed symbols. Results obtained with differently weighing portions of adipose tissue from individual animals are joined by lines.

To assess whether extracellular events participate in the catabolism of insulin in the fat pad system, leaching studies were performed (figure 5). Three differently weighing portions of right epididymal adipose tissue from three rats were incubated in media containing 0.1 milliunit of insulin per ml. and insulin degradation was measured at the end of ninety minutes of incubation (figure 5, left). Comparable portions of the left epididymal fat pads from these animals were incubated simultaneously without insulin. Thereafter, 1 ml. aliquots were removed from the latter systems and combined with 1 ml. of fresh medium containing sufficient insulin to yield a final concentration of 0.1 milliunit/ml. in the cell-free reaction mixtures. As shown on the right in figure 5, the cell-free systems effected negligible degradation of insulin. Thus, release of insulin-degrading enzymes into the suspending medium by adipose tissue could not be demonstrated with the leaching technic.

3. The effects of insulin concentration and normal human plasma on the degradation of insulin in in vitro bio-assay systems: The experiments presented above were all conducted at a single concentration of insulin (0.1 milliunit/ml.) that is well within the quoted range for normal fasting human plasma. To assess the effects of increased insulin concentrations, paired comparisons were conducted in which the insulin in the aqueous suspending media was augmented one hundredfold (table 1). At these insulin concentrations of 10.0 milliunits/ml., the percentage of the total insulin that was degraded was unaltered in the Randle system and only slightly reduced in the Groen preparation. On the other hand, the proportional breakdown of insulin was diminished in the Vallance-Owen and Martin preparations (table 1). In some of these experiments, the effects of plasma were evaluated concurrently. Specimens of plasma were obtained from fasting, normal laboratory personnel and added, as recommended in the individual assay procedures[10-15] (figure 1) to suspending media to yield mixtures containing a final concentration of 0.1 milliunit I^{131}-insulin/ml. In all systems, the presence of plasma was accompanied by a reduction in the percentage of I^{131}-insulin that was degraded (table 1). It does not appear likely that this phenomenon can be attrib-

TABLE 1

The effects of insulin concentration and normal, fasting human plasma upon insulin degradation during bio-assay in vitro

Assay system	Suspending medium*		Nonprecipitable I^{131} (Per cent of total I^{131}) Incubation: 10 minutes		Incubation: 90 minutes	
	Insulin (mU/ml.)	Plasma (ml./ml.)	N†	Per cent	N†	Per cent
Randle	0.1	0	3	26.6	3	82.2
	10.0	0		29.5		83.0
	0.1	0.25		21.6		76.3
Groen	0.1	0	3	18.4	3	75.3
	10.0	0		14.3		66.1
	0.1	0.16		9.3		61.4
Vallance-Owen	0.1	0	4	7.8	4	44.7
	10.0	0		4.7		36.7
	0.1	0.95		0.7		22.5
Martin et al.	0.1	0	4	1.2	11	13.1
	10.0	0		1.3		10.2
	0.1	0.95		0.2	(5)	5.9

* Suspending medium supplemented with insulin and plasma. Concentration of insulin expressed in terms of milliunits added per milliliter of final reaction mixture (mU/ml.). Plasma added as outlined in the individual assay procedure. Concentration of plasma expressed in terms of milliliter plasma per milliliter of final suspending medium (ml./ml.).

† N denotes the number of experiments in which the three situations were compared simultaneously. Per cent indicates the average percentage of the total insulin that was degraded in these experiments, after appropriate correction for TCA-soluble radioactivity in control vessels incubated without tissues for comparable intervals. Degradation in Martin et al. system expressed as percentage degraded per 100 mg. of fat pad. As denoted by the parentheses, the effects of plasma in the Martin system were assessed in only five of the eleven experiments.

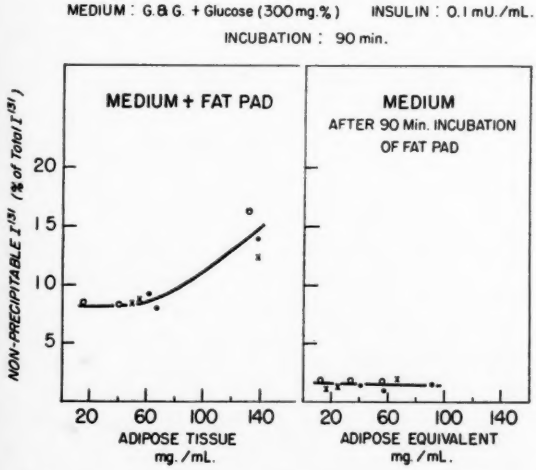

FIG. 5. Release of insulin degrading activity into the medium during incubation of rat epididymal fat pads: The symbols refer to individual values obtained with differently weighing portions of adipose tissue from three rats; mean values are indicated by the solid line. Results obtained during the standard ninety-minute incubation of insulin with adipose tissue are shown on the left. Insulin degradation during ninety-minute incubation of cell-free systems with insulin is illustrated on the right. Cell-free systems had been obtained by leaching adipose tissue from the three animals for ninety minutes. "Adipose equivalent" refers to the amount of adipose tissue which had been leached per milliliter of final reaction mixture. Weights are expressed on the basis of initial wet weight. For details, see text.

uted to the endogenous insulin of the plasma since in every paired experiment, a greater proportion of the total insulin was degraded in simple aqueous media even at insulin concentrations of 10.0 milliunits/ml. (table 1). The effects of plasma were least pronounced in the Randle and Groen systems where intrinsic insulin degrading capacities are great and only 0.25 or 0.16 ml. of plasma are added per milliliter of medium. The effects of plasma were most pronounced in the Vallance-Owen and the Martin systems where intrinsic insulin-degrading capacity is low and 0.95 ml. of plasma are added per milliliter of final suspending solution.

DISCUSSION

The present studies have demonstrated the occurrence of appreciable degradation of insulin in four of the major systems for the in vitro bio-assay of insulin. The data prompt certain methodological and theoretical considerations.

It has been shown that insulin proteolysis is greatest in the systems in which cut hemidiaphragms are employed and that the magnitude of insulin breakdown is conditioned by the number of suspended hemidiaphragms (Randle > Groen >> Vallance-Owen). Therefore, attempts to increase the biological sensitivity of these procedures by increasing the mass of insulin-responsive tissue entail the attendant risk of augmented insulin degradation. In the diaphragm systems, degradatory phenomena may be facilitated by the release of

proteolytic enzymes from cut muscle fibers. Such leaching is minimal with adipose tissue, and here, insulin degradation may be determined by rates of cellular penetration as well as intracellular proteolytic activity. Nonetheless, in the fat pad as in the hemidiaphragm, a relationship exists between tissue weight and insulin breakdown. Thus, for standardized bio-assay, it may be desirable to limit mass to a narrower range than the 80 to 200 mg. of adipose tissue[15] that has been proposed.

Under the conditions recommended for the individual bio-assays, the system of Martin and others effected the least degradation of insulin. Such comparison of bio-assay procedures was the intent of the present studies. It should be noted, however, that the manifestly low level of insulinolytic activity in adipose tissue may be more apparent than real because of the dilution of tissue weight by triglycerides. Thus, absolute comparison of the insulin degrading potential of adipose tissue and other structures should be on a nitrogen basis.

Intrinsic to all bio-assay procedures are the assumptions that (a) the assay portion of the dose-response curve reflects a constant relationship and (b) the unknown specimen does not contain factors which may alter the standard dose-response relationships. The present data provide relevant information in both areas.

First, it has been demonstrated that a smaller percentage of the total insulin in aqueous media is degraded as the concentration of insulin is increased from 0.1 to 10.0 milliunits per ml. As might be anticipated, the effects were inversely related to the insulin degrading capacities of the individual systems (Martin > Vallance-Owen > Groen > Randle). The fact that the proportional breakdown of insulin is affected by the concentration of insulin would suggest that a constant correction factor cannot be employed to correct aqueous bio-assay standards for insulin degradation over this one hundredfold range of concentration. Whether such correction would be justified over narrower bio-assay ranges cannot be determined from present data.

Secondly, it has been demonstrated that normal, fasting plasma inhibits insulin degradation in all of the bio-assay systems. In the least, this observation indicates that unknown specimens of plasma and known aqueous standards are not completely comparable in any of the systems. Could correction be made for the effects of plasma? Although the presence of plasma theoretically should result in a closer approximation of the true and observed dose-response relationships, such phenomena would have to be examined at multiple concentrations of insulin. Moreover, the factors in normal plasma which inhibit insulin degrading enzymes are relatively specific and largely concentrated in Cohn Fractions III and IV-1.[9] Hence, a constant potential for inhibition need not obtain in abnormal plasmas. The present findings that plasma caused least change in the Randle system (where the medium is supplemented with 25 per cent plasma), and was most inhibitory in the Vallance-Owen and Martin preparations (where the medium is supplemented with 95 per cent plasma) only support the prediction that the effects of plasma upon insulin proteolysis in vitro will be conditioned by (a) the insulin-degrading capacity of the assay tissue, and (b) the quantity of plasma employed in the assay.*

Within the accepted bio-assay range for normal plasma insulin, the contributions of degradation would not be very pronounced. For example, even with 50 per cent degradation, a true value of 0.10 milliunit/ml. would be reduced to only 0.05 milliunit/ml. However, the apparent "smallness" of this difference does not detract from its significance but rather attests to the relative crudeness of bio-assay, and the relatively large range of "permissible" error. The present studies were designed to focus upon one possible source of this variability with the hope that future inquiry may elucidate many other factors which must be contributory also.

It should be mentioned that all of the above extrapolations from the available data contain the tacit implication of a prolonged stoicheiometric relationship between unaltered insulin and insulin action. This need not be the case. The possibility cannot be excluded that insulin in vitro initiates an immediate change in the assimilitative capacities of responsive tissues, and that the magnitude and duration of this effect are conditioned by the initial concentration of insulin rather than by its subsequent fate. However, this possibility seems less likely on the basis of recent data. Narahara and Williams[20] have demonstrated a potentiation of the effects of small quantities of insulin in the rat diaphragm system by enriching suspending media with protein hormones which retard insulin degradation. Moreover, work in this laboratory has demonstrated that the response of surviving rat adipose tissue to suboptimal doses of insulin is reduced when the system is immediately supplemented with soluble insulin-degrading enzymes which do not, per se, appreciably alter the metabolism of adipose tissue.[22] Nevertheless, whether the *continuing* availability of extracellular insulin is necessary for *sustained* insulin action during *prolonged* incubation has still to

*These phenomena presumably bear little relevance to another aspect of plasma dilution described by Randle;[21] namely, an apparent enhancement of insulin-like activity by serial dilution of plasma.

be answered. In any event, for the moment, it would appear that the present experimental findings and implications merit consideration and reservation in interpreting the quantitative results of in vitro bio-assay.

SUMMARY

I^{131}-insulin has been employed to assess the magnitude of insulin degradation during in vitro bio-assay performed according to the rat diaphragm procedures of (a) Randle, (b) Groen and Willebrands, (c) Vallance-Owen and Hurlock, and (d) the rat adipose tissue method of Martin, Renold, and Dagenais. Appreciable degradation of insulin occurs in all systems. Degradation is greatest in the cut muscle preparations and proteolytic enzymes are leached into the suspending medium. Insulin breakdown is least in the adipose tissue preparation and leaching phenomena are minimal. In all systems, the proportional degradation of insulin is conditioned by the mass of incubated tissue and by the extracellular concentration of insulin. Moreover, in all systems, the degradation of insulin is significantly inhibited by factors present in normal plasma. Theoretical implications of these findings are discussed.

SUMMARIO IN INTERLINGUA

Re-Evalutation del Methodos de Bio-Essayage pro Insulina in Vitro

Insulina marcate con I^{131} esseva usate pro evalutar le magnitude del degradation de insulina durante le bio-essayage in vitro effectuate con le technicas a diaphragma de ratto de (a) Randle, (b) Groen e Willebrands, (c) Vallance-Owen e Hurlock, e (d) con le technica a histo adipose de ratta de Martin, Renold, e Dagenais. Appreciabile grados de degradation de insulina occurre in omne le systemas mentionate. Le degradation es le plus marcate in le preparatos de musculo secate ab que enzymas proteolytic es extrahite a in le medio de suspension. Le degradation de insulina es le minus marcate in le preparatos de histo adipose ab que le extraction es minimal. In omne le systemas le degradation proportional de insulina es conditionate per le massa de histo incubate e per le concentration extracellular de insulina. In plus, in omne le systemas il occurre un inhibition del degradation de insulina como effecto de factores que es normalmente presente in le plasma.

ACKNOWLEDGMENT

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Influence of Glucose and Tolbutamide in the Fasted Animal

Ralph G. Janes, Ph.D., Hal A. Alert, B.S., and Norman D. Paul, B.S., Iowa City

A voluminous literature has appeared in the last three or four years on the effect, in animals and human beings, of certain sulfonylurea derivatives. These compounds, chiefly tolbutamide and carbutamide, produce marked hypoglycemia in the normal animal or human being,[1-4] but have little effect in the severely diabetic.[3-6] The mechanism whereby the sulfonylurea compounds produce a hypoglycemia is becoming clearer. Apparently they cause release of granules, presumably containing insulin, from the beta cells of the pancreatic islands.[4,7-10] In fact, some investigators have found the effects noted in the normal animal, after receiving these oral hypoglycemic compounds, to be very similar to those following the administration of insulin.[11,12]

The influence of the sulfonylurea compounds on liver glycogen is of particular interest. If an intact unfasted animal is treated with insulin[13] or tolbutamide[11] the liver glycogen levels do not seem to be altered. However, fasted animals given insulin[14] or sulfonylurea compounds,[1,11,12,15] show increased levels of liver glycogen. Apparently the latter effects may be caused, at least in part, by a decreased liver glycogenolysis which has been reported for animals treated with sulfonylureas.[16-19]

After sulfonylurea therapy, the pancreatic beta cells of normal animals show various degrees of degranulation.[10,20,21] Glucose also produces some degranulation of these cells.[22] Because of the similar action of glucose and tolbutamide on beta-cell granulation, the present study was undertaken to compare the effects of these materials when given alone or in combination. Alterations in blood sugar and liver glycogen levels also have been studied.

MATERIALS AND METHODS

Adult rats of the Long-Evans strain were fasted for two days and divided into four groups. Each group received, at each feeding by stomach tube, one of the following: (1) 2.5 ml. water, (2) 1.85 gm. of glucose/200 gm. body weight dissolved in 2.5 ml. water, (3) 83.3 mg. of tolbutamide*/200 gm. body weight dissolved in 2.5 ml. of water, or (4) a combination of glucose and tolbutamide dissolved in 2.5 ml. of water. All of the animals were given two feedings on the third fasting day and those that were studied for four days were given an additional three feedings. The feedings were spaced as widely apart as possible; i.e., for the experiments with two feedings, one feeding was given in the evening and another the next morning. For the experiments with five feedings, the two feedings were repeated and the fifth feeding was given in the afternoon of the last fasting day. Blood sugar determinations were made one and one-half hours after the last feeding on the third or fourth fasting days. Blood sugar levels were determined by the methods of Nelson-Somogyi and liver glycogen by the method of Montgomery.[23] The pancreas was fixed in Bouin's fluid, and stained by Gomori's aldehyde-fuchsin technic after oxidation with potassium permanganate. An estimation of the beta-cell granulation was made without the identity of the material being known to the observer. The granulation was rated from 0 to 4 plus, 4 plus representing maximum granulation. The unabsorbed glucose in glucose-fed animals was determined in samples of the stomach and small intestine which were homogenized for one minute in a Waring Blendor. It was possible to recover approximately 75 per cent of the glucose with this procedure and no correction was made for glucose not recovered in the final tabulation for unabsorbed glucose. A Fiske osmometer was used to determine the osmolarity of the solutions which were fed.

RESULTS

The usual hypoglycemic response to tolbutamide was noted in animals fasted two days and given tolbutamide on the third fasting day (figure 1). In fact, with the dose used, many rats had such low blood sugar levels that they showed signs of muscular weakness although there was no evidence of hypoglycemic convulsions. Moreover, when rats were given glucose by stomach

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*Supplied as the sodium salt by The Upjohn Company, Kalamazoo, Michigan.

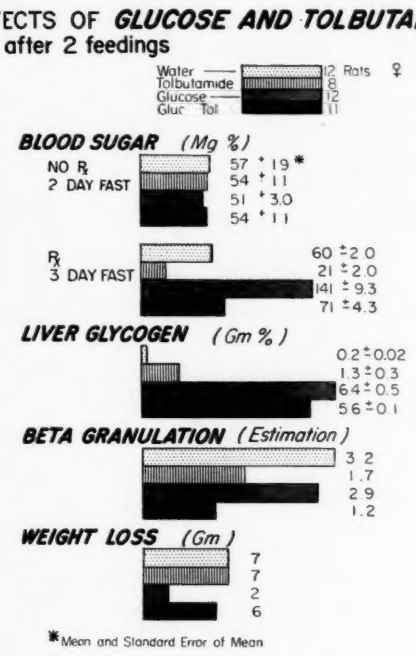

FIGURE 1

tube, there was a marked increase in blood sugar, but tolbutamide prevented most of this elevation (figure 1).

Liver glycogen values were of particular interest in these animals. Control rats fed only water the last day of the three-day fasting period had very low liver glycogen levels. When tolbutamide dissolved in water was substituted for water, however, there was a sixfold increase in liver glycogen over the control level. On the other hand, glucose feeding produced a large increase in liver glycogen values, but the addition of tolbutamide failed to produce an additional increase in liver glycogen; in fact, the average values were a little lower than when glucose only was given (figure 1).

In view of the alterations in carbohydrate levels following administration of glucose and/or tolbutamide in these rats, the granulation of the beta cells of the pancreatic islets was examined. The beta cells of the islets of the control animals receiving only water were well filled with granules. Tolbutamide caused a marked reduction in the granulation. On the other hand, glucose appeared to produce some degranulation, whereas the combination of glucose-tolbutamide caused more degranulation than tolbutamide alone (figure 1).

The animals showed a greater response to glucose and/or tolbutamide when they were given five feedings, i.e., two on the third fasting day and three more on the fourth fasting day (figure 2). The water-fed animals maintained rather constant blood glucose levels during the second, third and fourth fasting days. The depression in blood sugar levels with tolbutamide and the elevation with glucose were similar on the third and fourth fasting days. However, the blood sugar was not altered in glucose-fed animals when tolbutamide was also fed, if the rats were given five feedings of these materials.

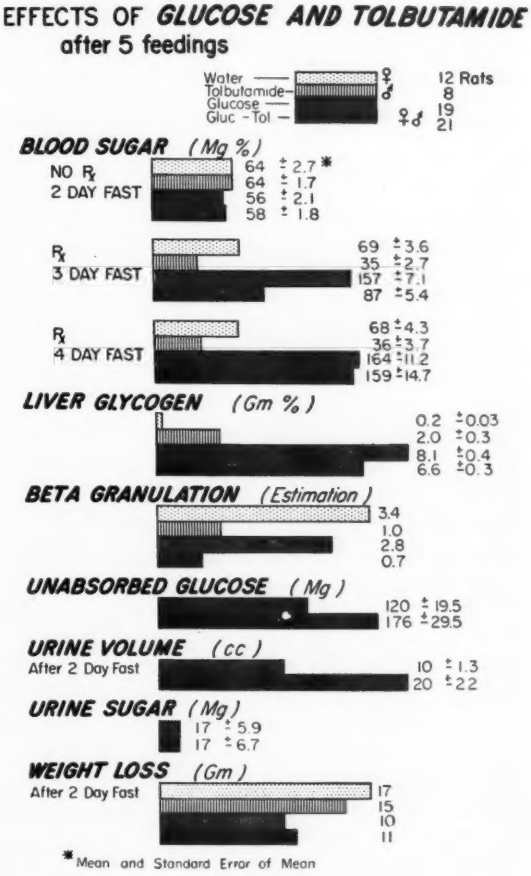

FIGURE 2

The changes in liver glycogen were more marked in the animals receiving five feedings. Tolbutamide caused a tenfold increase over the water-fed controls. On the other hand the average liver glycogen values for glucose-tolbutamide-fed animals appeared to be lower than when only glucose was given.

The granulation of the pancreatic beta cells appeared a little more abundant in the rats receiving five feedings of water than in those receiving two feedings (figure

2). Moreover, tolbutamide caused a more marked degranulation when given five times than when given two times. The degranulation caused by five feedings of glucose was about the same as when two feedings were given but there was a nearly complete degranulation when five feedings of glucose and tolbutamide were given. Examples of the beta-cell granulation following the various experimental procedures are shown in figure 4.

In observing the animals which received five feedings of glucose it was apparent that considerable fluid still was present in the stomach one and one-half hours after the last feeding. Indeed, the animals did retain some glucose in the stomach and small intestine and this was particularly evident in those animals which received both glucose and tolbutamide (figure 2). Since there is a possible relationship between the osmotic pressure and retention of glucose in the stomach and small intestine,[34] the osmolarity of the solutions was tested. The effective mOsm/L. was 235 for the solution of tolbutamide, 3,075 for the glucose and 3,235 for the glucose and tolbutamide. Furthermore, because of the high blood glucose levels in some animals, the question arose whether they were excreting glucose in the urine. The glucose-fed animals excreted some glucose in their urine but this represented only a small fraction of the amount fed. During the third and fourth days of the fast, the rats excreted relatively small amounts of urine but the volumes were larger in the glucose-tolbutamide-fed animals than in those fed glucose only.

The amount of weight lost by the rats following the initial two days of fasting was observed, as a possible factor in altering blood sugar and liver glycogen levels. The rats receiving only water lost the most weight. This was surprising because the rats receiving only tolbutamide had higher liver glycogen levels and one would suspect that there would be less protein breakdown in these animals. Glucose when given alone or with tolbutamide prevented some of the weight loss seen in the other two groups of animals.

It was somewhat disappointing to encounter such large variations in glucose and glycogen values in the rats fed glucose. Part of these variations were caused by differences in the rate glucose was absorbed by different animals. However, it appeared that perhaps the sex of the animals might be a contributing factor. Consequently, the responses of male and female rats to glucose and glucose-tolbutamide were compared after five feedings of these materials (figure 3).

In general, the blood sugar levels of the females during the third and fourth fasting days were higher than

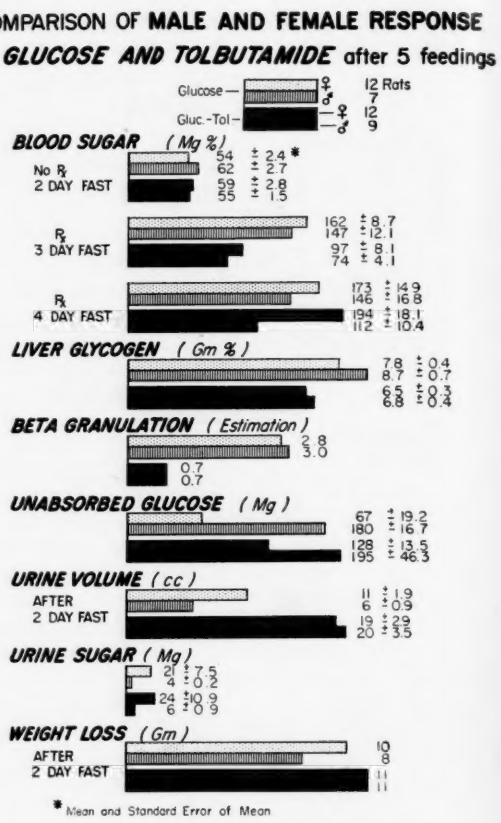

FIGURE 3

those found for the males. Moreover, the liver glycogen levels appeared to be a little lower in the females than in the males. Also, the unabsorbed glucose values were higher in the males than in females, but the females excreted more glucose in their urine, although the latter values were so small they wouldn't alter, to any great extent, the total carbohydrate present in the body. The urine volumes or weight loss did not appear to be different. The beta-cell granulation also failed to give any information as to possible sex differences.

DISCUSSION

The degranulation observed in the beta cells of the pancreatic islets in tolbutamide- and glucose-fed rats appears to be caused by two independent factors. The degranulation occurring after the administration of glucose is probably a response of the beta cells to hyperglycemia. On the other hand, it is still not clear why the sulfonylureas cause degranulation. Presumably, both

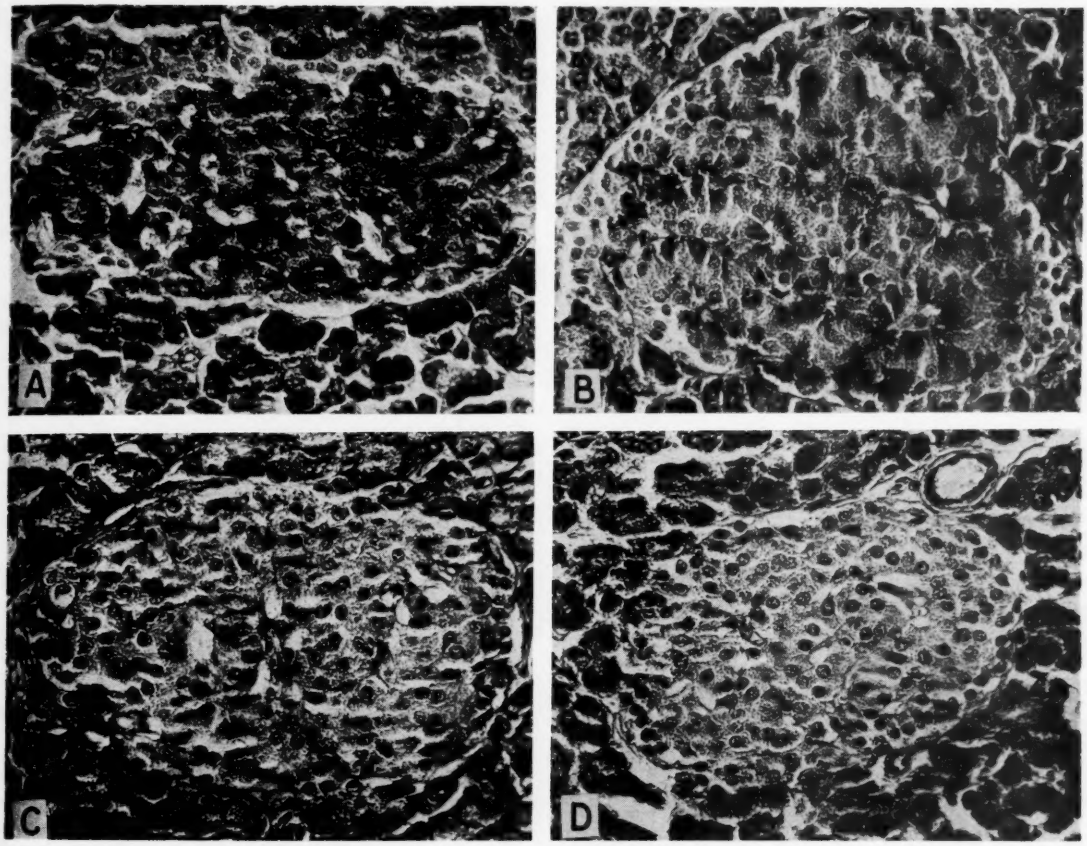

FIG. 4. Effect of various procedures on the beta cells of the pancreatic islets. All tissues shown are from rats fasted for two days and given a total of five feedings during the third and fourth fasting days.
A. Islet from control rat fed water. Estimation of beta-cell granulation 3.5.
B. Islet from rat fed glucose. Estimation of beta-cell granulation 2.5.
C. Islet from rat fed tolbutamide. Estimation of beta-cell granulation 1.0.
D. Islet from rat fed glucose and tolbutamide. Estimation of beta-cell granulation 0.5.

factors affecting degranulation work independently when tolbutamide and glucose are given simultaneously, since degranulation achieved under these conditions approximately equals the sum of the degranulation caused by each compound when given separately.

While considering degranulation of the beta cells following the administration of sulfonylurea compounds, it seems worth while to note that two investigators have not found this degranulation.[5,25] Furthermore, Volk et al.[20] found only mild and sporadic degranulation the first three days of treatment with tolbutamide, but observed a marked degranulation of the beta cells on the fourth day. In the present experiments a marked degranulation of beta cells was seen in all pancreases from rats treated with tolbutamide for one or two days. However, it has been found that if pancreas tissue is stored in fixative or alcohol for two or three days the beta granules are dissolved partially or entirely and will not stain properly with the aldehyde-fuchsin technic. Perhaps some of the differences concerning degranulation observed by other investigators may be associated with the way the staining technics have been carried out.

It was not possible to work out any association between blood sugar and liver glycogen levels with the degree of beta-cell granulation. It is true that in fasting animals given tolbutamide there is always a hypoglycemia and an increase in the level of liver glycogen. However, as soon as glucose or the combination of glucose and tolbutamide is given, degranulation of the

beta cells occurs but there is no relationship between this degranulation and the carbohydrate levels studied. For instance, glucose per se causes some degranulation of the beta cells and an increase in both blood sugar and liver glycogen levels. On the other hand, when five feedings of glucose and tolbutamide are given, blood sugar values are not altered when compared with glucose controls, although beta cells show the greatest amount of degranulation. Moreover, in such animals the liver glycogen is elevated but the values are not so high as when only glucose is given.

A discouraging part of this study is the variation seen in glucose and glycogen levels in the rat following glucose feedings, whether glucose is given by itself or with tolbutamide. It appears that at least part of the variation can be accounted for by two facts. First, there are apparent differences in the absorption rate of glucose in male and female rats. The females appear to absorb glucose more rapidly than the males. Second, tolbutamide when fed with glucose slows down the absorption of glucose in many animals. These latter rats show a marked variation in their ability to absorb glucose. It has been pointed out that sulfonylurea compounds may delay the absorption of glucose from the alimentary tract,[30] although other workers[27,28] have failed to find such an effect.

SUMMARY

Adult rats of the Long-Evans strain were fasted for two days and divided into four groups. Each group was fed by stomach tube: water, glucose, tolbutamide or glucose and tolbutamide. All the animals were given two feedings on the third fasting day and some were given three additional feedings on the fourth fasting day.

Tolbutamide caused a decrease in blood sugar levels and an increase in liver glycogen. Glucose feeding produced a large increase in blood sugar and liver glycogen values. When two feedings of tolbutamide and glucose were given, the blood sugar was lowered to near control levels and the liver glycogen was lower than when only glucose was given. However, after five feedings of glucose and tolbutamide the blood sugar levels were similar to those found in animals given only glucose.

Both glucose and tolbutamide caused a degranulation of the beta cells of the pancreatic islets. Apparently this effect was produced by different mechanisms. When glucose and tolbutamide were combined the total degranulation was at least equal to the degranulation produced by each compound separately.

Considerable variation was noted in the blood sugar and liver glycogen levels of animals fed glucose and the combination of glucose and tolbutamide. This was caused partly by differences in the response of males and females to these compounds. Also, some variation was caused because tolbutamide apparently delayed the absorption of glucose from the alimentary tract.

SUMMARIO IN INTERLINGUA

Effecto de Glucosa e Tolbutamido in Animales Jejunate

Rattos adulte del racia Long-Evans esseva tenite jejun durante duo dies e dividite in quatro gruppos. Le animales in le quatro gruppos esseva alimentate per tubo gastric con aqua, glucosa, tolbutamido, o glucosa e tolbutamido, respectivemente. Le animales esseva alimentate in iste maniera duo vices le tertie die post le initio de lor jejunation e tres vices le quarte die.

Tolbutamido causava un reduction del sucro de sanguine e un augmento del glycogeno hepatic. Glucosa produceva un grande augmento del sucro del sanguine e del glycogeno hepatic. Quando duo repastos de tolbutamido e glucosa esseva administrate, le sucro del sanguine descendeva a nivellos proxime al nivellos de controlo, e le glycogeno hepatic esseva plus basse que quando glucosa sol esseva administrate. Tamen, post cinque repastos de glucosa e tolbutamido, le nivellos del sucro de sanguine esseva simile a illos trovate in animales recipiente solmente glucosa.

Tanto glucosa como etiam tolbutamido causava un disgranulation del cellulas beta in le insulas del pancreas. Apparentemente iste effecto esseva producite per differente mechanismos. Quando glucosa e tolbutamido esseva combinate, le disgranulation total esseva al minus equal al disgranulation producite per le un e le altere composito individualmente.

Variationes considerabile esseva notate in le nivellos de sucro sanguinee e glycogeno hepatic de animales tractate con glucosa e con le combination de glucosa e tolbutamido. Isto esseva causate in parte per differentias in le responsas de masculos e de femininas. In plus, un parte del variation esseva causate per le facto que tolbutamido apparentemente retardava le absorption de glucosa ab le vias alimentari.

ACKNOWLEDGMENT

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It is impossible to understand the detailed mechanism of the mode of action of any pharmacologically active substance on the basis of its effects in the intact animal; conditions are far too complex.

Progress towards the elucidation of the mode of action of pharmacologically active substances is made when their effect can be reproduced in isolated organs, tissue slices or tissue homogenates; it then becomes sometimes possible to localize the drug effect on definite enzyme systems. Several of the vitamins have in this way been recognized as essential components of enzyme systems, for instance vitamin B_1 as cocarboxylase and vitamin B_6 as the coenzyme for transamination. No such success has as yet been attained in the case of the hormones, which may be due to the fact that their action is bound up with the structural organization of the cell. However, clear-cut in vitro effects on isolated tissue or tissue slices have been obtained with several hormones, and insulin was the first hormone for which such an effect was firmly established.

From "Present Knowledge of the Mechanism of the Mode of Action of Insulin," by E. B. Chain, A. Beloff-Chain and F. Pocchiari, in *Transactions of the Istituto Superiore di Sanità,* Vol. 1, part 3, 1956, Rome, page 409.

EDITORIALS

The Editorial Board of this Journal, the Committee on Scientific Publications and the Council of the American Diabetes Association share the loss of our distinguished Editor, scientist and friend, Dr. William C. Stadie, with his family, his colleagues at the University of Pennsylvania and the scientific community of the world. Elsewhere in this issue our able President, longtime friend and co-worker of Dr. Stadie, has presented a story on his life.

The Editorial Board and his Associate Editor are grateful for the few years he was able to serve the Journal and the American Diabetes Association. As Editor, his impeccable scientific standards and gentle but firm leadership have left an imperishable mark in the history of the Journal.

THE CLINICAL TESTING OF ORAL HYPOGLYCEMIC AGENTS

The chief requirements of any oral preparation for the treatment of diabetes are two: (1) it must be safe and (2) it must be at least as good as insulin for patients who need therapy in addition to diet. Specifically, we shall interpret the second requirement to mean that the oral agent must be as capable as insulin of maintaining the blood sugar below 150 mg. per 100 cc. and the urine free of sugar or nearly so with the patient on a diet appropriate to his ideal body weight and activity.

Although the responsibility for evaluating new remedies rests originally with their industrial developers and ultimately with the Food and Drug Administration, there is an in-between stage in which the fate of such products depends upon the results of preliminary clinical trial. It is, after all, the judgment of the physician that labels a drug safe or toxic, effective or useless. The decisive importance of his observations demands that he employ experimental designs as foolproof, and criteria as rigid, as those of the laboratory worker.

From this point of view, the office- or clinic-treated diabetic makes a poor test subject. Variations in food intake, activity, emotional stress and motivation almost preclude the controlled conditions that ought to surround the exploration of a new form of treatment. These variables account in part for the unreliability of some of the first reports on the sulfonylureas by clinicians who worked mainly with ambulatory patients. Such patients have their place in clinical trials, but that place is later rather than earlier.

The first clinical testing of a new drug for diabetes is best done in the hospital, preferably the metabolic ward. Even here, special precautions must be observed. Of first importance is the selection of cases. Obese patients should be avoided, for the vast majority of them, when placed on a proper diet in the hospital, will be found not to need insulin even though they have seemed to at home; and the purpose of the study is to determine whether the drug in question can replace insulin that is actually required. Patients with newly discovered diabetes, who tend to improve with any kind of treatment, or those whose tolerance for carbohydrate is changing for other reasons such as a recent illness or surgical operation, should not be chosen. The ideal subject is the otherwise healthy individual with diabetes of one year's duration or more, a well established requirement for insulin, an unchanging body weight and a stable emotional make-up.

Diet, obviously, must be constant. The caloric allowance should be calculated to maintain the customary body weight in the condition of reduced activity that hospitalization entails.

Except in special circumstances, insulin should be withdrawn rather than reduced, for withdrawal, but not reduction, will tell whether insulin was necessary at all and, if so, whether the oral drug can completely replace it. There is not much point in ascertaining whether the test preparation can compensate for the loss of 10 units out of a customary 20 unit dosage, for example, because, first, the apparent ability to alter that elusive entity, "insulin requirement," is an unreliable measure of a drug's activity and second, because there is little to be gained by using an oral supplement in the routine management of patients for whom some insulin is necessary.*

The withdrawal of insulin should be followed not by the immediate administration of the test substance but

* The claim, not fully established, that some oral hypoglycemic agents minimize the fluctuations of the blood sugar in patients with labile diabetes might be considered an exception to this statement.

by a *control period* of at least ten days during which fractional and twenty-four-hour urine specimens are analyzed daily for glucose and, if positive, for ketone bodies and both fasting and postprandial blood sugar levels are determined each day. If blood and urinary glucose remain within acceptable limits as defined above, the patient needs neither insulin nor oral drug and has proved himself an unsuitable subject.

If hyperglycemia and glycosuria appear or continue during the control period, the drug to be tested is added to the regimen, with no other change, for the next ten days (*test period*), during which comparable data are collected and signs of toxicity watched for.

A *recovery period* of similar length terminates the observations.

If during the test period normal or nearly normal glucose levels are achieved, it can be concluded that the compound is effective for the patient in question and possibly for others of a similar type. If good control is not obtained, the compound is not as good as insulin for this patient and the opposite conclusions can be drawn.

Ideally, a period with a placebo instead of the test substance should follow the recovery period, but this adds materially to the time required and is far less important in hospital than outpatient observations.

Obviously, this elaborate and time-consuming kind of study is not suitable for large numbers of patients or for the determination of long-term toxicity. Its purpose is to provide unassailable data on the effect of a given preparation in a few carefully selected subjects with diabetes of various types and degrees of severity. Only after the accumulation of this sort of information should the base of clinical testing be broadened to include ambulatory cases.

With respect to office or clinic patients, we are not concerned here with single-dose response tests of a few hours' duration but with the effectiveness and safety of drugs on a maintenance basis. The procedure follows the general scheme just presented but with two important modifications: Insulin must be withdrawn slowly rather than abruptly in order to guard against ketosis, and the "double-blind" method of administering the test drug and a placebo is imperative. The inherent difficulties of this kind of observation are mitigated to some extent by longer control, test and recovery periods; weekly visits at which blood sugar and quantitative (twenty-four-hour) urinary glucose are determined; written records of home urine tests made four times daily; and measurements of body weight.

Short-cuts in these procedures have been taken too often in the past, and the tendency to take them is currently increasing with the successive introduction of new forms of the sulfonylureas and other hypoglycemic agents. The fact that a new drug differs from its predecessor by only an atom or two is no guarantee that either its biologic activity or its safety is identical. Witness the example of carbutamide, in which merely the substitution of a methyl for an amino group converted that compound to one (tolbutamide) which is appreciably less toxic and lacks bactericidal properties while retaining essentially the same hypoglycemic potency. In the interest of sound medical practice and public safety, and despite the pressure of commercial competition, each new compound, however similar to others, deserves the same careful clinical testing as if its close relatives had never been heard of.

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THE TUBERCULOUS DIABETIC

The publication of a report on the management of the tuberculous diabetic by Luntz[1] has prompted this comment. Its purpose is to emphasize again a concluding statement by Luntz, to wit, "Acute bilateral pulmonary tuberculosis in a diabetic is a serious complication to be regarded as a medical emergency. It calls for immediate treatment in a special hospital unit with an effective dosage of combined chemotherapy for the tuberculosis and the stabilization of the diabetes as a matter of great urgency."

The validity of these conclusions cannot be questioned by any one who has had the opportunity to observe these cases. The tragic consequences of neglect of both diseases and, per contra, the remarkable success of combined therapeutic efforts in the past twenty years testify to the correctness of the conclusions.

The experience of Luntz was based, it is true, on only a short (one year) follow-up of eighty-four cases. He noted the usual sequence, tuberculosis complicating pre-existent diabetes in the majority of instances. The age distribution was also the usual one, with two peaks of predominance, twenty to thirty-nine and forty-five to sixty-four years, the first seeming to correspond to the maximal incidence of tuberculosis, the latter to the maximal incidence of diabetes. It would seem unnecessary to remind any practitioner again, in these days of mass X rays, of the dictum of at least yearly chest X rays in all diabetic patients and in particular in those with a break in tolerance or poor or difficult control. Such vigilance is imperative in the light of Luntz's finding of bilateral

disease in over half of his cases, in 68 per cent three or more zones involved and in 87 per cent cavitation present when first seen. This bespeaks advanced and profuse involvement. It signifies either delay or neglect either by the patient or his medical attendant. This is not a unique experience. It is noted in all institutions where these patients are segregated.

Dr. Luntz followed the recommendation of the Ministry of Health for special provision for tuberculous diabetic patients and organized a hospital unit, out-patient clinic and domiciliary service, thus providing continuity of treatment and supervision. Such ideal arrangements are not common by any means, although it is in operation at least in one of New York's institutions and should be in all others. This organization paid dividends. There was only one death in the ensuing year and this was not due to tuberculosis. Granted that one year is an insufficient period to assess the progress of tuberculous patients, the achievement of quiescence of disease in 83 per cent speaks for itself. The success of this program also seems evident from several indices: Reduction from an incidence of about 90 per cent positive sputum examinations to 6 per cent positives after one year; disappearance of cavitation in 83 per cent; decreases in sedimentation rate and pyrexia and gain in weight. These results compare favorably with those reported in nondiabetic tuberculous patients.[2]

The treatment program for the tuberculous infection was the usual combined chemotherapy, bed rest, collapse therapy including pneumothorax, pneumoperitoneum with and without phrenic nerve crush, thoracoplasty and lobar and segmental resection. Major surgery was well tolerated. The regulation of diabetes during and after the operative procedure followed conventional lines. The excellent results underline the fact that a tuberculous diabetic patient should be treated for the tuberculosis as thoroughly by all medical and sugical modalities as if his diabetes did not exist. But his diabetes must be controlled concomitantly so that he is as close to nutritional balance as possible. The Birmingham Group used measured diets to accomplish this, usually 2,500 calories, C—275, P—110, F—110, varying with the assessed nutritional requirements. These correspond closely to our ADA standard dietaries. Insulin, both soluble and depot, was given in various combinations, and distinct efforts were made to keep these patients aglycosuric and as nearly normoglycemic as possible.

The excellent results of this combined program of intense therapy for both diseases are similar to the experience of others.[3] It is my impression that the progressive improvement in prognosis over the years may be correlated better with the advancements in the therapy of tuberculosis than with changes in diabetes therapy. A final opinion on this point awaits a statistical analysis of a large series of patients. This is not to deny, by any means, the fundamental relationship between stabilization of the diabetic state and stabilization of the infection. No one will deny the grave prognosis to the infected individual without this control of his metabolic abnormality.

But the basic problem of the susceptibility of the diabetic to infection is still unsolved. The incidence of tuberculosis in diabetic subjects is several times that of the general population of similar age, sex, race and social status. Conversely, there seems to be little doubt that susceptibility to tuberculosis is decreased in the stabilized diabetic patient. This statement becomes more meaningful if we conceive of tuberculosis, according to Bloch,[4] as an intercellular, dormant, symbiotic infection awaiting the right combination of alterations in the "microbiological environment of inflammation" (Dubos)[5] to become active. The experiments of Dubos[6] on the effect of the nutritive state on this environment, particularly the effect of ketosis, strengthen this concept for the diabetic subject.

The lethal effect of artificially induced changes in the tricarboxylic acid cycle in Salmonella-infected mice demonstrated by Berry,[7] may be cited as another experimental example perhaps particularly applicable to the diabetic's metabolic dysfunction. Many such factors, nutritional, neural or endocrine-regulatory, biophysical and biochemical are being studied throughout the world and it would be naive, indeed, to select any one of them as the ultimate determinant of the diabetic patient's susceptibility.

As Bloch[4] has put it for tuberculosis: "During the chronic phase, the tuberculous infection remains stationary. Despite the continuous presence of living infectious organisms, clinical signs of tuberculosis are lacking. The animal (or man) has acquired a considerable degree of immunity against new infections, but at the same time he is liable to an exacerbation of his own condition after having been exposed to a variety of seemingly unrelated impacts such as nutritional or endocrine changes, or intercurrent infections other than tuberculosis."

These statements illustrate the acquired or phenotypic characteristics of disease and the numerous adaptations of both host and parasite necessary for the survival of either. But one must consider the genotype of both host and parasite. Natural resistance to infection is difficult

even to define and has proved much more difficult to assess. As yet, no specific alteration in any of the systems of natural resistance such as the Properdin one has been detected in the diabetic.

However, this does not mean that some alteration in an essential system, inherited concurrently and separately with his inherited metabolic abnormality, may not be present in the diabetic patient. If one considers that "genes control the potentiality of enzyme syntheses" (Spiegelman and Campbell)[8] then it does not seem too speculative to envisage a subtle inherited alteration in the enzymatic synthesis of the diabetic host's responsive metabolites when confronted by the aggressive metabolites of the parasite. Induced enzyme formation in the microorganism (Spiegelman and Campbell)[8] and metabolic adaptations in the mammalian organism (Knox)[9] must be conditioned by the genetic structure of both.

To express it in the words of Snyder,[10] "Each individual has a unique assembly of genes and will have his own mode of reaction to disease, whether it be presented through infection, trauma, stress or malnutrition, or wholly from within through biochemical error. The conviction that the genetic constitution is involved to a greater or lesser extent in all disease will serve as a stimulant to look beyond the secondary aspects of pathology and to search for the primary genic action in each case."

It remains to be seen whether the genotype of the diabetic may play an essential role in his peculiar susceptibility to infections other than through his known inherited metabolic abnormality. All we may state with any degree of confidence at present is that the intrinsic inherited defects in metabolism that characterize the diabetic state are factors in his susceptibility to infection.

The inescapable conclusion from all this would seem to be that these defects in his metabolism must be remedied or controlled to the best of our ability if the diabetic is to escape his predestined tendency to infection or to be rescued if infection occurs.

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BOOK REVIEW

THE LIPIDS: THEIR CHEMISTRY AND BIOCHEMISTRY. *Vol. III, by Harry J. Deuel, Jr. $25, pp. 1065, Interscience Publishers, Inc., New York, 1957.*

This is the third volume of Dr. Deuel's comprehensive treatise on the chemistry of the lipids. The present volume is published posthumously and together with its predecessors forms a fitting monument to the genius and indefatigable labors of Dr. Harry J. Deuel, Jr. The present volume is concerned with the biosynthesis, oxidation, metabolism, and nutritional value of lipids. There are fourteen chapters comprising some 1,000 pages. This book is an indispensable reference for biochemists working in this complex field. Chapter I deals with the problems of the digestion, absorption, transport and storage of lipids. Chapter II is a masterly detailed account of the biosynthesis of various forms of lipids in the animal body. It serves as a most valuable exposition of the development and the present status of this field. Chapter III covers the problems in oxidation and metabolism of the triglycerides, phospholipids, and fatty acids in the animal body. Chapter IV is an able exposition of the vexed question of the conversion of fat to carbohydrate. Deuel comes to the balanced conclusion that a net increase of carbohydrate from fat does not occur in the mammalian organism. The phospholipids are treated with respect to oxidation and metabolism in Chapter V. The role of the low molecular weight fatty acids, such as acetic, formic and propionic, in the intermediary metabolism of fat is treated in Chapter VI. The biochemistry of unusual forms of fatty acids, such as branch chain, hydroxy, and keto acids, etc., is treated in a separate chapter. Excellent and complete discussion of the sterol chemistry comprises some 100 pages in Chapter VII. The biochemistry of the fat soluble vitamins is treated in Chapters IX to XII. The discussion here is most thoroughgoing, of historical significance, and presents the modern developments most succinctly. Chapter XIII deals with the interesting biochemistry of the essential fatty acids, and the final chapter concerns itself with the nutritional value of fats. There is an excellent author and subject index. This particular volume of a series of three deserves high rank among the present available treatises on the rapidly developing field of lipid chemistry.

William C. Stadie
1886—1959

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William Christopher Stadie died on Sept. 11, 1959, at the age of seventy-three. In the course of a life marked by the affection of family and friends, he was a great man, an outstanding scientist and a beloved guide to his companions in research. Because he will receive many tributes, it seems fitting that this memorial should emphasize the part he played in the growth of our knowledge of diabetes and in the combined service and leadership which he contributed as editor of this Journal and as an active participant in many activities of the American Diabetes Association.

For the record, a conventional curriculum vitae *is included. Dr. Stadie was born in New York on June 15, 1886, and graduated from the College of Physicians and Surgeons (Columbia) in 1916. After interning at the Presbyterian Hospital in New York, he moved to The Rockefeller Institute Hospital, 1918-22; was Assistant Professor of Medicine at Yale University from 1922 to 1925, when he came to the University of Pennsylvania as Associate Professor of Research Medicine. From 1941 to 1956 he was John Herr Musser Professor of Research Medicine and Emeritus Professor from 1956 until his death in 1959. Since 1956 he served as Editor of* DIABETES. *He was a member of the American Society of Biological Chemists for which he served on the editorial board of their journal and as treasurer; the Association of American Physicians; American Society for Clinical Investigation; Harvey Society; American Diabetes Association; National Academy of Sciences and American Philosophical Society.*

His achievements were recognized by the following awards: The John Phillips Memorial Medal of the American College of Physicians (1940); the Kober Medal of the Association of American Physicians (1955); the Banting Medal of the American Diabetes Association (1956); the Alvarenga Award of the College of Physicians of Philadelphia (1957); and the Honorary Degree of Doctor of Science from the University of Pennsylvania (1959).

Underlying this biographical compilation are the accomplishments for which his friends and fellow scientists admired him and from which they learned so much.

His publications from The Rockefeller Institute describe the development and use of arterial puncture for the study of respiratory and cardiac diseases; the analytical methods for determining blood gases; the construction of the first quantitatively controlled oxygen chamber and its use in the treatment of pneumonia. The historic stimulus to this work was the influenza epidemic of 1918, but thousands of pneumonia patients have since benefited. Today, in large hospitals, oxygen is piped to each bedside and blood gas analyses are routine procedures.

At Yale and the University of Pennsylvania there followed (1924-39) a series of articles on blood gases, acid-base balance and the development of a fundamental comprehension of the physical chemical aspects of these important functions of blood.

From 1939 until his death Stadie was engaged in studies on the metabolism of fat and carbohydrate and on the action of insulin and he wrote more than ninety reports and lectures in this field of biochemistry. From the outset his results were dramatic. His address at the time of the Phillips Award[1] in 1941 concluded:

"The diabetic who, by reason of insulin lack, is unable to utilize carbohydrate to the full measure of his metabolic needs, must fall back on fat for his energy requirements. Part of this need is met by the initiation and completion of fat oxidation in the muscles themselves. However, a considerable fraction, estimated at one third to one half of the total caloric needs from fat, is obtained by a preliminary oxidation of fats in the liver to ketone bodies only. Neither acetic acid nor glucose is formed by this oxidation. These hepatic ketone bodies are freely utilized for energy by the periphery without insulin and without the necessity of simultaneous carbohydrate oxidation. This reserve mechanism, however, appears to be incapable of fine regulation so that when the demand for fat calories exceeds a certain level, approximately 2.5 gm. of fat/kilogram/day

for the resting state, ketone bodies in excess of needs are formed by the liver. The excess is excreted as ketones in the urine. If this excessive fat catabolism continues unchecked, ketosis and coma follow."

Only those who endured the pre-Stadie confusion in this area can appreciate the magnitude of his contribution. Those who read his more extensive expositions[2,3] can understand the careful quantitative methods and painstaking correlation of his results with those of others, which gave substance to these conclusions. Even now, after the introduction of isotopic methods, the quotation published eighteen years ago remains the modern concept of fat metabolism in diabetes.

In the course of these studies, he also evolved new theories of the chemistry of fat oxidation. The old theory was that fats were burned by beta oxidation of the long-chain fatty acids, with splitting off of successive molecules of acetic acid. These were oxidized until the chain was shortened to four-carbon acids, which in the absence of glucose oxidation, were excreted as ketone bodies. This meant that one molecule of each fatty acid could yield only one molecule of ketones. Stadie showed that four molecules of keto acids were derived from one molecule of palmitic acid. Without disproving beta oxidation as a possibility, he pointed out that acetyl groups from fatty acids could be recombined into four-carbon acids. With minor modification by isotopic methods, this remains today's basic concept.

At this stage of his work, the second World War was in progress, and Stadie undertook investigations on oxygen poisoning. When oxygen is breathed at one or more atmospheres, convulsions and other damaging effects occur. With Riggs and Haugaard, a series of ten papers on the effects of high oxygen pressures on various tissues, enzymes and intact mice revealed again his classic thoroughness. Readers will rightly ponder the engineering skill which produced these studies of tissue at physiological temperatures but at pressures that required completely original apparatus.

In 1950 he broadened his investigation of the effects of insulin by examining its influence on different tissues and various substrates. In particular, he demonstrated the combination of insulin with those tissues in which its in vitro effects were best demonstrable, such as muscle and adipose tissue, but not the brain which is independent of insulin. This demonstration was made,

Oxygen chamber of The Rockefeller Institute while in use in 1920

first by dipping the tissue in a solution of insulin for five minutes or less, washing the tissue copiously and then observing the full insulin effect in the next few hours. Secondly, the use of isotopically labeled insulin enabled him to establish its attachment to well washed tissues. In 1954 he gave a detailed account of the current status of our knowledge of the action of insulin.[4] However, the more brief but masterful summary which this Association heard and read in his Banting Lecture[5] in 1956 will remain a landmark for all of us.

All of Stadie's contributions required "a rare combination of abilities, chemical, physiological, clinical, and engineering."[6] His insistence on exact quantitation and his superb training in mathematics and physical chemistry formed the firm basis for his discoveries in biology and medicine. To the end he retained his brilliant logic and originality with undiminished vigor. As Van Slyke[6] said: "Some statisticians have tried to prove that an investigator's peak is reached at forty, after which his curve of productivity falls with the accelerating descent of a spent cannon ball. Stadie, among other contributions, has proved that when science remains the mistress of one's heart and mind, the peak of production can in truth be a plateau that continues its high level all through the years of life."

Outside of the laboratory, his life was marked by varied interests, strong friendships and by a richly happy family life. He played the cello and the clarinet, enough to understand and delight in classical music, was a critical and liberal thinker about civic matters and at any lunch hour could make his companions better citizens. He had a good workshop in his home and was a really expert carpenter and cabinetmaker. He knew and enjoyed gardening, bee keeping and all such activities of a simple home. Best of all, the satisfactions of sincere love and affection showed through all the joys and sorrows of his family life. Above all his scientific achievements rise the love and gratitude of his friends for the lifetime he spent helping them.

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In searching for a theory to explain the mode of action of insulin the main facts to be considered are that all metabolic reactions which are stimulated by insulin are of synthetic nature, and that the insulin effect is an immediate one. The synthetic reactions accelerated by insulin are glycogen synthesis, fat synthesis, protein synthesis and growth, the nature and extent of the reactions depending on the tissue and the conditions. In muscle tissue glycogen synthesis from glucose and amino acid incorporation into protein are accelerated, in liver and mammary gland fat synthesis, in the brown adipose tissue both glycogen and fat synthesis, and in tissue culture explants the general synthesis of cell material.

From "Present Knowledge of the Mechanism of the Mode of Action of Insulin," by E. B. Chain, A. Beloff-Chain and F. Pocchiari, in the *Transactions of the Istituto Superiore di Sanità,* Vol. 1, part 3, 1956, Rome, page 507.

ABSTRACTS

Abrén, Kurt (Institute of Physiol., Univ. of Lund, Sweden): EFFECT OF INSULIN ON BODY GROWTH AND MAMMARY GLAND DEVELOPMENT IN FORCE-FED HYPOPHYSECTOMIZED RATS INJECTED WITH OVARIAN HORMONES. Acta endocrinol. 30:593-615, April 1959.

Carefully controlled experiments were performed on hypophysectomized rats to study the effect on weight gain, linear growth and mammary gland development of factors such as ad libitum or tube forced feeding, and oestrone, progesterone and insulin administration. Forced feeding alone was largely responsible for weight gain in oestrone-treated rats; insulin added little. Linear growth was not affected.

Mammary gland development of significant degree required both oestrone and insulin administration, the latter in doses higher and for a longer duration than in previous growth experiments in this preparation. Progesterone particularly encouraged alveolar gland development. S.B.B.

Bailey, J. Martyn (Finney-Howell Cancer Res. Lab., Dept. of Surgery, Johns Hopkins Med. School, Baltimore, Md.): A MICROCOLORIMETRIC METHOD FOR THE DETERMINATION OF SORBITOL, MANNITOL, AND GLYCEROL IN BIOLOGIC FLUIDS. J. Lab. & Clin. Med. 54:158-62, July 1959.

The author describes a rapid microcolorimetric method for the determination of sorbitol, mannitol, and glycerol in biologic fluids. The procedure employs periodate oxidation followed by a colorimetric determination of the formaldehyde formed, and is suitable for 5 to 50 mg. quantities of material. Glucose and other blood or serum components do not interfere, and the method may be used for the dual determination of glucose and the polyhydric alcohols present in a single sample. G.J.H.

Batts, Adrienne A.; Gaal, Stephen A.; and Tipton, Dale L., Jr. (Dept. of Physiol. & Anat., Univ. of California, Sch. of Med., Berkeley, Calif.): CHANGES IN THE GOLGI APPARATUS OF THE ISLETS OF LANGERHANS IN THE RAT FOLLOWING GLUCOSE AND INSULIN ADMINISTRATION. Endocrinology 64:503-12, April 1959.

The importance of a criterion of cellular activity is well recognized. The appearance of the Golgi apparatus provides information on the secretory activity of cells. Alterations in the Golgi apparatus of pancreatic beta cells may be more helpful than granule counts since the latter represent a balance between production and release.

Glucose infusion into rats results in an increase in size and complexity of the Golgi apparatus while insulin infusion causes an involution. Insulin-induced hypoglycemia produced no changes in the alpha cell Golgi apparatus. R.L.J.

Beaser, Samuel B. (Harvard Med. Sch. and Beth Israel Hosp., Boston, Mass.): THERAPY OF DIABETES MELLITUS WITH COMBINATIONS OF DRUGS GIVEN ORALLY. New England J. Med. 259:1207-10, Dec. 18, 1958.

A combination of phenethyldiguanide with tolbutamide or chlorpropamide was administered orally to ten diabetic patients who had been unsuccessfully treated with tolbutamide. The detailed results in four such patients are reported. There was an additive effect in three, with complete replacement of insulin in two. No toxic effects of such combined therapy were noted during one to four months of treatment. W.R.K.

Beatty, Clarissa H.; Peterson, R. D.; Bocek, Rose Mary; and West, Edward S. (University of Oregon Medical School, Portland, Ore.): ACETOACETATE AND GLUCOSE UPTAKE BY DIAPHRAGM AND SKELETAL MUSCLE FROM CONTROL AND DIABETIC RATS. J. Biol. Chem. 234:11-15, January 1959.

Diaphragm and adductor muscle from alloxan diabetic rats exhibited a decreased uptake of acetoacetate and glucose as compared to muscle from control rats. Glucose uptake was increased by the addition of insulin to adductor muscle. Therefore, decreased peripheral utilization of ketone bodies probably contributes to diabetic ketosis. A.R.C., JR.

Bennison, B. E. (Med. Res. Div., Esso Res. and Engineering Company, Linden, N.J.): HYPOGLYCEMIC AGENT TOXICITY. J. Occupational Med. 1:401-03, July 1959.

The clinical usefulness of the presently approved oral hypoglycemic agents seems likely to usher in a new era in diabetic therapy. Although the pendulum is currently near the peak of optimism, significant side effects involving predominantly the liver, gastrointestinal tract, and skin are being reported. Physicians in industrial medicine will want to bear these side effects in mind as they periodically review the health status of diabetic employees. W.R.K.

Berry, Maxwell G. (St. Luke's Hosp., Kansas City, Mo.): TOBACCO HYPOGLYCEMIA. Ann. Int. Med. 50:1149-57, May 1959.

This is a report of twenty-four patients (twenty-two men) with symptomatic hypoglycemia attributed to tobacco smoking and seen over a period of thirteen years. The hypoglycemia did not occur principally in the fasting state. In many patients a distinct hypoglycemic response occurred as a late development in the glucose tolerance test and the profile of the latter was distinctly raised after cessation of smoking. All patients smoked more than a package of cigarettes per day and cessation of smoking eliminated the syndrome. Illustrative cases are described. S.B.B.

Berryman, G. H. (University of Illinois Coll. of Medicine, Chicago, Ill.): NEWER CONCEPTS OF OBESITY. Illinois M. J. 115:74-76, February 1959.

Derangements of physiological control mechanisms in the production of obesity are discussed. Included are experimental hypothalamic disturbances and the presence of a fat-mobilizing factor in the serum and urine of fasting human subjects. However, the treatment of obesity at present still requires attention to the patient's social and emotional problems, caloric and nutritional requirements and physical activity appropriate to the individual. H.L.W.

Bothe, Frederick A. (Philadelphia, Pa.): SURGICAL PROCEDURES IN THE DIABETIC PATIENT. Postgrad. Med. 25: 437-42, April 1959.

The diabetic patient who requires an operation is a challenging problem; there is no phase of medicine in which teamwork between the surgeon and the endocrinologist is of greater importance than in the management of such patients. Many diabetic patients will go through operations without much difficulty, but in a large number electrolyte imbalance and difficulties will develop which make the operative procedure more risky, and morbidity of recovery of these patients is propor-

tionately higher than of the nondiabetic patients. The diabetic patient is no greater risk operatively than the condition of his cardiovascular-renal tree, if given thorough evaluation by laboratory examinations and sufficient time for proper management of diabetes. W.R.K.

Brown, Donald W. (Dept. of Internal Med., The Med. Group, Honolulu, Hawaii): TREATMENT OF DIABETES MELLITUS WITH TOLBUTAMIDE: REVIEW OF THIRTY CASES. Hawaii M. J. *18*:245-48, January-February 1959.

Tolbutamide is indicated and can apparently be used safely in present-day clinical practice provided certain careful limitations are observed. The patients should be very carefully selected. They should be mild diabetics needing 10 to 30 units of insulin daily. They should be thirty-five years old or older. There should be no history of acidosis. Once selected, they should be very carefully followed. Frequent blood sugars and blood counts are necessary at first. The same regimen followed by diabetics on insulin should be observed; that is, a careful diet, frequent urine tests and visits to the doctor. W.R.K.

Burt, Richard L. (Dept. of Obstetrics and Gynecology, Bowman Gray Sch. of Med. of Wake Forest College, and the North Carolina Baptist Hosp., Winston-Salem, N.C.): REACTIVITY TO TOLBUTAMIDE IN NORMAL PREGNANCY. Obst. & Gynec. *12*:447-53, October 1958.

As with insulin, resistance to the hypoglycemic effect of tolbutamide develops in pregnancy. The hypoaminoacidemia developing after administration of tolbutamide is qualitatively comparable to that following insulin administration but is exaggerated in pregnancy. This finding is believed to be unrelated to the effect on blood sugar. W.R.K.

Buschmann, G.; Fritze, E.; and Marsch, A. (Medizinische Universitatsklinik, Kirchweg 1, Göttingen, Germany): LONG-TERM FOLLOW-UP OF 1,500 DIABETICS. German M. Month. *4*:10-14, January 1959.

Over 70 per cent of diabetics die nowadays of arteriosclerotic vascular lesions, the so-called long-term diabetic vascular syndrome. In order to investigate the cause of these fundamental changes and to draw conclusions regarding treatment, the case histories, physical and laboratory findings, and the progress of 1,547 diabetic patients were recorded by a punch-card system and analyzed. The patients had been under continuous medical care for an average of four (one to eleven) years. The incidence of the long-term vascular syndrome, which includes first of all diabetic retinopathy, was found to be 30 per cent and rose to 70 per cent in cases of diabetes of over twenty years' duration. Fifty-three per cent of the patients were overweight, 11 per cent underweight. The obese and those in poor metabolic balance were particularly liable to show vascular lesion. These were formed in 34 per cent of overweight and 18 per cent of underweight cases, and in 43 per cent of poorly balanced ones, as opposed to 19 per cent of those who were always in good metabolic control. Of cases of over twenty years' duration, the incidence of vascular complications was 77 per cent in those poorly or variably controlled and 40 per cent in those always well balanced. Pulmonary tuberculosis, other infections, and liver lesions were more frequent among poorly controlled patients and also among those who suffered from a more serious form of diabetes. Although the incidence of vascular lesions rose progressively as the duration of diabetes increased, much can be done to prevent or delay this process by careful metabolic balance through diet and insulin, and by aiming at a low body weight. W.R.K.

Collens, William S.; Silverstein, Joseph N.; and Dobkin, Gerald B. (Maimonides Hosp. and Med. Dept., State Univ. of New York, Downstate Med. Center, Brooklyn, N.Y.): CASE OF A DIABETIC WITH A KIMMELSTIEL-WILSON SYNDROME AND A NORMAL GLUCOSE TOLERANCE. Ann. Int. Med. 50: 1282-88, May 1959.

This paper reports a patient with diabetes and a Kimmelstiel-Wilson syndrome, whose capacity to handle carbohydrate was normal by standard glucose tolerance tests. Although these tests were normal by both the oral and the intravenous routes, the first sign of any abnormality was seen when the patient was exposed to stress by both steroids and infection. The interesting feature presented by this patient was the disappearance of the diabetes as far as its recognition by his capacity to utilize glucose was concerned. In spite of this he developed a profound form of unequivocal diabetic nephropathy and retinopathy as documented by renal biopsy and fundus photographs. It would appear from this that the disturbance in carbohydrate metabolism which occurs in diabetes as a result of a disordered insulin function may be independent of the mechanism responsible for the nephropathic and retinopathic changes often seen in the diabetic. There is probably some other mechanism in the body, completely divorced from a disturbance in insulin metabolism, which is participating in the production of nephropathy, retinopathy and even neuropathy. Certainly a patient with what appears to be a normal insulin function who yet develops nephropathic and retinopathic changes would indicate that there are two separate systems operating in the diabetic, and that a disturbance in the function of insulin as it affects the metabolism of carbohydrate and fat is only one facet of the over-all picture of diabetes mellitus. W.R.K.

Constam, G. R.; Leemann, W.; and Almasy, F. (Medical Policlinic consultations for diabetics; Medical clinic of the veterinary faculty; Chemical research laboratory of the veterinary faculty; University of Zurich, Switzerland): ABOUT THE EXCRETION OF ZINC IN THE URINE OF DIABETICS. Schweiz. med. Wchnschr. *88*:1103-05, November 1958.

In 1938 Scott and Fisher suggested that zinc might be necessary for the normal storage of insulin in the pancreas. By injecting zinc-binding substances, such as diphenylthiocarbazone, Kadota produced diabetes in rabbits. Wolff and co-workers discovered a transitory increase in the urinary zinc elimination following intravenous injections of diphenylthiocarbazone and also after the injection of alloxan.

Twenty-four-hour specimens of human urine were treated by wet digestion and measured colorimetrically for zinc according to the method of Vallee and Gibson.

The urinary zinc elimination of thirty-eight nondiabetics varied from 39 to 892 μg. per twenty-four hours with an average of 337 and the standard deviation of 207.8 μg. per twenty-four hours. Determination of the daily zinc losses in forty diabetics resulted in values from 394 to 5,845 μg. per twenty-four hours with an average of 1,203 and the standard deviation of 914.3 μg. per twenty-four hours. The difference between the mean diabetic and the mean nondiabetic zinc loss, 866 μg. per twenty-four hours, is statistically highly significant. Severe decompensation of the diabetes is usually accompanied by a considerable increase in zinc elimination through the urine. The rise in zinc output apparently does not run parallel to diuresis, glycosuria, ketonuria nor blood sugar concentration; it seems unrelated to the age of the patient or the estimated

duration of the disease. Generally zinc excretion grows smaller with better control of the diabetes.

It remains to be clarified whether the increase in urinary zinc elimination in uncontrolled diabetes is the cause or the consequence of the diabetic disorder or just an accompanying symptom. (German). M.G.G.

Danforth, Elliot, Jr.; and Moore, Richard O. (Ohio State Univ., Dept. of Agricultural Biochem., Columbus, Ohio): INTESTINAL ABSORPTION OF INSULIN IN THE RAT. Endocrinology 65:118-23, July 1959.

Diisopropyl fluorophosphate (DFP) along with amorphous zinc-free insulin was injected into the jejunum of rats. The DFP promoted the absorption of about one two-thousandth of the injected insulin presumably by inhibiting the proteolytic effect of trypsin and chymotrypsin.

Soybean trypsin inhibitor failed to promote insulin absorption. Indole-3-acetate, a liver insulinase inhibitor, given intraperitoneally prior to the intrajejunal injection of DFP and insulin increased the hypoglycemic effect of the insulin.

Insulin passed through the intestinal wall of an everted sac preparation regardless of the presence of DFP since in this preparation proteolytic enzymes are removed by washing. H.L.W.

Davis, T. W.; Kerr, R. B.; and Bogoch, A. (Dept. of Med., Shaughnessy Hosp. and Univ. of British Columbia, Vancouver, B.C.): EXPERIENCE WITH GLIPASOL (R.P. 2259)—AN ANTIDIABETIC SULFONAMIDE DRUG. Canad. M. A. J. 81:101-07, July 15, 1959.

The effects of an antidiabetic sulfonamide drug, R.P. 2259 or Glipasol, were studied in thirty-one patients with maturity-onset diabetes mellitus. This drug is one of the sulfonamide drugs upon which the investigations of Loubatières and his colleagues were made in France during and after the second World War. It differs from carbutamide and tolbutamide in that it possesses a thiodiazole rather than a urea nucleus and has a tertiary rather than a normal butyl end prosthetic group. It also resembles carbutamide and differs from tolbutamide in having an amine group substituted on the benzene ring. Thirty-one adult male patients with maturity-onset diabetes mellitus were given the drug. These patients ranged from forty-six to eighty years of age, had been known to have diabetes for from a few weeks to eighteen years, and had been controlled with from 15 to 90 units of insulin daily; all except four had been easily controlled previously with insulin. Glipasol satisfactorily controlled the blood sugar levels in twenty (65 per cent) of the patients. The effectiveness of Glipasol was unrelated to age (all patients were over forty-five years). It was less effective in patients who had known diabetes for ten years or more, in those who had received insulin therapy for ten years or more, in those who required more than 50 units of insulin daily, and in those whose diabetes had previously been difficult to control with insulin. Seventeen, or 54 per cent, of the patients developed laboratory evidence of hepatic dysfunction while receiving Glipasol. Liver biopsy in five patients with jaundice showed hepatitis characterized by parenchymal involvement and portal and periportal inflammation. This drug is therefore considered to be too toxic for clinical use. W.R.K.

Dobrzanski, T. (Lomzynska 8, Zyrardov, Poland): THE INFLUENCE OF TOLBUTAMIDE (D-860) ON THE LIVER PARENCHYMA IN DIABETES MELLITUS. German M. Month. 4:229-30, July 1959.

The existence of histologic and functional liver damage in diabetes mellitus has been recognized for a long time. The favorable effect of adequate control of the diabetes upon liver changes and the enhanced sensitivity to insulin after improvement of liver function in the diabetic have both been described. This paper reports the results obtained when testing the effect of tolbutamide upon parenchymal liver damage in the course of diabetes mellitus. No significant correlation was established between the degree of morphologic changes in the liver cells, the results of the tolbutamide tolerance test, and the liver function tests. The reported observations suggest that it would be desirable to determine whether the morphologic liver changes in patients who continue to respond to tolbutamide are similar to those found in patients who have developed secondary failure to tolbutamide therapy after a short period of response, or whether the secondary failure of response to tolbutamide is related to the histologic changes found in the second and third groups of patients described in this report. W.R.K.

Editorial (Boston, Mass.): SIGNIFICANCE OF INSULIN-RESISTANT DIABETES. New England J. Med. 261:98, July 9, 1959.

Many observations have been made indicating that patients treated with insulin may develop antibodies. In most cases, these antibodies are not antagonistic to insulin action; however, some cases of insulin resistance may be explained by insulin binding in the blood. Studies such as that reported by Leonards and Martin indicate the existence of another type. In a case of insulin resistance, they demonstrated that the patient's blood contained a large amount of "insulin-like activity." Evidently, the insulin administered to the patient accumulated in her blood, but could not be utilized in her tissues. Similar observations have been made in a small number of other cases. If easy and accurate methods for estimation of the insulin content of the blood were available, they might be widely used in the diagnosis and treatment of diabetes. Although all methods employed up to this time are highly technical and not available for general application, the work of Leonards and Martin illustrates how selective use in a problem case can contribute valuable information to the physician and physiologist. W.R.K.

Feinberg, Arthur W.; and Lax, Henry (Great Neck, N. Y.): ABNORMALITIES OF THE ARTERIAL PULSE WAVE IN YOUNG DIABETIC SUBJECTS. Circulation 18:717, Part 2, October 1958. (Abstract of the paper presented at the 31st Scientific Sessions of the American Heart Association, S.F., Oct. 24, 1958.)

In patients with peripheral arteriosclerosis and hypertension, abnormalities have been noted in the arterial pulse wave, recorded by the authors with a new technic from digits of human subjects. Arterial pulse waves were taken on thirty-one diabetic subjects between the ages of ten and twenty-nine years, and twenty-two, or 67 per cent, were found to have abnormal arterial pulse waves, although none of these twenty-two patients had any clinical evidence of arteriosclerosis or hypertension. There was no correlation between the severity of the diabetes and the presence of abnormal arterial pulse waves. The longer the duration of the diabetes, the more likely pulse wave configurations would be abnormal.

Eighty-eight per cent of patients who had diabetes longer

than five years had abnormal tracings. In twenty-one control patients with a positive family history of diabetes, eleven were found to have abnormal pulse waves.

This technic, according to the authors, seems to offer a means of detecting changes in the arterial pulse-wave configuration of young diabetics, and in nondiabetic subjects with a positive family history of the disease, that antedates clinical evidence of change in their vascular system. R.C.C.

Freinkel, Norbert; and Ingbar, Sidney H. (Harvard Med. Sch., Boston, Mass.): THE EFFECTS OF THE ARYLSULFONYLUREAS ON THE RESPIRATORY ACTIVITY AND GLUCOSE METABOLISM OF ISOLATED RABBIT KIDNEY CORTEX. Endocrinology *64*: 1002-09, June 1959.

Orinase reduced oxygen consumption and increased cellular hydration in slices of rabbit kidney cortex. Glucose in the incubating medium fell. The incorporation of labeled glucose into lipids and CO_2 diminished. The amount of Orinase required exceeded by several times the plasma levels which are therapeutically effective in vivo. These findings may represent an isolated toxic effect or alternatively a localized "Pasteur effect" which may shed light on how Orinase acts. H.L.W.

Gastineau, Clifford F.; and Power, Marschelle H. (Section of Medicine and Section of Biochemistry, Mayo Clinic, Rochester, Minn.): METABOLIC STUDIES OF A PATIENT WITH OSTEOPOROSIS AND DIABETES MELLITUS. Proc. Staff Meet. Mayo Clin. *34*:165-72, April 1, 1959.

A thirty-four-year-old man who had had diabetes mellitus for nineteen years and disabling pain in the back for two years was found to have an extensive demineralizing bone disease best described as osteoporosis. Metabolic studies failed to show any clinically significant relationship between the state of diabetic control and calcium balance. W.R.K.

Gittler, Robert D.; Fajans, Stefan S.; and Conn, Jerome W. (Dept. of Internal Medicine, University of Michigan Med. Sch., Ann Arbor, Mich.): COEXISTENCE OF ADDISON'S DISEASE AND DIABETES MELLITUS: REPORT OF THREE CASES WITH A DISCUSSION OF METABOLIC INTERRELATIONSHIPS. J. Clin. Endocrinol. *19*:797-804, July 1959.

The total number of reported cases of coexisting Addison's disease and diabetes mellitus has now reached seventy. Up to 1949, a review of the literature revealed only twenty-four such cases. The increasing number reported during the past ten years is undoubtedly a reflection of more frequent clinical recognition of Addison's disease. In most instances, the diabetes has preceded the adrenal insufficiency. In such cases the ameliorating effect of adrenocortical insufficiency upon diabetes mellitus has been striking. The situation is analogous to that demonstrated first by Long and Lukens in the depancreatized adrenalectomized cat. Adrenocortical insufficiency should be suspected whenever a diabetic patient manifests a sharp decrease in total insulin requirement together with great instability of the blood sugar level and severe hypoglycemic episodes. Resistance to ketosis and hypoglycemia unresponsiveness are confirmatory findings. Despite amelioration of the diabetic state, the patient shows the progressive deterioration characteristic of untreated Addison's disease. Administration of physiologic amounts of carbohydrate-active steroids restores the previous intensity of the diabetic state, resulting in a sharp rise in the concentration of blood sugar, increased urinary loss of sugar, increased insulin requirements, diminished insulin sensitivity, increased nitrogen excretion, and a return of susceptibility to ketosis. Although replacement therapy for adrenal insufficiency in the patient with coexisting Addison's disease and diabetes mellitus produces intensification of the diabetic state, it imparts greater stability and results in easier management of the diabetes. W.R.K.

Goodman, Joseph I. (Diabetic Clinic, Mt. Sinai Hosp., Cleveland, Ohio): THE DIETARY MANAGEMENT OF DIABETES. Ohio M. J. *55*:800-04, June 1959.

The author presents a brief, concise, and yet very realistic and practical approach to the dietary management of diabetes using the accepted exchange technics as sponsored by the American Diabetes Association and the American Dietetic Association. G.J.H.

Grodsky, G. M.; and Peng, C. T. (Met. Unit for Research in Arthritis & Allied Diseases, Dept. of Med., & Radioactivity, Univ. of California Sch. of Med., San Francisco, Calif.): EXTRACTABLE INSULIN MEASURED BY IMMUNO-CHEMICAL ASSAY: EFFECT OF TOLBUTAMIDE. Proc. Soc. Exper. Biol. & Med. *101*:100-03, May 1959.

The authors describe the application of a new method for immuno-chemical assay for extractable insulin from the pancreas based on the change in binding of insulin tagged with I^{131} to insulin antibodies in the presence of various quantities of unlabeled insulin. This method was used to study changes in extractable pancreatic insulin after administration of tolbutamide to normal mice. Although tolbutamide caused a sharp decline in blood sugar within two hours of administration, a consistent decrease of insulin in the pancreas was not observed. G.J.H.

Haunz, E. A.; and Cornatzer, W. E. (Univ. of North Dakota, Sch. of Med., Grand Forks, N.D.): PRESENT-DAY THERAPY OF DIABETIC KETOACIDOSIS AND COMA IN GENERAL PRACTICE. Minnesota Med. *41*:836-42, December 1958.

A practicable approach to the therapy of diabetic ketoacidosis and/or coma within the limited facilities of the general practitioner is presented. Certain basic fundamentals of good medical and nursing care are omitted in the interest of brevity and because such information is readily available in standard textbooks. Lack of sufficient insulin is the common denominator of the numerous metabolic complexities of diabetic acidosis and coma. Probably the commonest source of error in treatment is underestimation of immediate insulin requirement. Suggestions are proposed to avoid or minimize this error. A simplified approach to the correction of profound electrolyte disturbances incident to diabetic ketoacidosis and/or coma has been proposed which should avoid the grave hazards of miscalculation when administering parenteral fluid and electrolytes to correct deficits in fluid volume, alkali reserve, sodium, and potassium. The calculations presuppose access to determinations of plasma carbon dioxide combining power, chlorides, and blood glucose. When such minimum facilities are not available, the physician should restrict treatment to emergency measures and transport the patient to the nearest medical center so equipped. To attempt therapy with inadequate facilities is foolhardy heroism which courts disaster, since diabetic coma remains a grave medical emergency with a mortality ranging from 3 to 15 per cent or higher depending upon the clinician's definition of diabetic coma. W.R.K.

Helander, Einar (Allergy Clinic, Sahlgrenska Sjukhuset, Gothenburg, Sweden): ASTHMA AND DIABETES. Acta med. scan-

dinav. *162*:165-74, Oct. 22, 1958.

The material for study comprises 3,151 patients with asthma and 3,236 with diabetes mellitus. The morbidity figures agree with those in the general population. One and fourteen hundredths per cent of the patients with asthma have diabetes and 1.10 per cent of the diabetic patients have asthma. Attacks of asthma are not accompanied by alterations of the blood carbon dioxide combining power of glucose levels. Coincidence of the two disorders is rare before the age of forty. Adrenalin did not affect the blood glucose or insulin requirement in patients who had the two diseases concomitantly. ACTH permanently aggravated the diabetes in two cases. B.P.K.

Hellman, Bo (Histological Dept., Univ. of Uppsala, Sweden): THE RELATION BETWEEN AGE AND THE β/α CELL RATIO IN THE ISLET TISSUE OF THE RAT. Acta endocrinol. *31*:80-90, May 1959.

The ratio between the number of β and α cells has been estimated in sixty male rats at ages ranging from the newborn to 480 days. The animals were reared on a diet containing 38 calories per cent carbohydrate, 24.5 calories per cent fat and 37.5 calories per cent protein. In the newborn rats relatively more α cells were observed than in adult animals. Later in life the β/α cell number ratio was surprisingly constant except in the case of the oldest animals. The relative decrease in β cells in this oldest age group is similar to the decreased ratio reported in the literature to have been found in man. The β/α cell number ratio has been compared in large and small islets. It can be stated that the small islets contain relatively more β cells than the large islets. The dominance of β cells in the small islets was most pronounced in the adult animal. W.R.K.

Hellman, Bo (Histological Dept., Univ. of Uppsala, Sweden): THE VOLUMETRIC DISTRIBUTION OF THE PANCREATIC ISLET TISSUE IN YOUNG AND OLD RATS. Acta endocrinol. *31*:91-106, May 1959.

The equations derived by Wicksell for the relation between the size distribution of the section surfaces and the actual volume distribution of spherical and ellipsoid bodies have been used in order to calculate the distribution of the islet volume in seventy-five male rats of ages varying from the newborn to 480 days. The animals were reared on a diet containing 38 calories per cent carbohydrate, 24.5 calories per cent fat and 37.5 calories per cent protein. The volume frequency curves for all age groups appeared to have a normal distribution, and this was confirmed by transformation to probits. Irrespective of age, it could be stated that in individual rats a larger islet volume in general caused a displacement of the normal curve towards the larger islet classes, i.e., to the right. If the comparison is limited to animals within the same age class, this still holds on the average. The increase of islet volume with age is manifested by an increase of the volumes in all classes, but especially in the classes with large islets. The resulting right shift in the volume frequency distribution curve was, however, less than would be expected from the increase in total islet volume. W.R.K.

Hellman, Bo; and Hellerström, Claes (Histological Dept., University of Uppsala, Sweden): DIURNAL CHANGES IN THE FUNCTION OF THE PANCREATIC ISLETS OF RATS AS INDICATED BY NUCLEAR SIZE IN THE ISLET CELLS. Acta endocrinol. *31*:267-81, June 1959.

This is a study of the functional activity of the beta and alpha cells of the rat in relation to time of day. The index used was nuclear size. Sixty male rats were used, ten being sacrificed at four-hourly intervals with free access to food and water at all times. The beta cell activity showed maximum peaks at twelve midday and midnight, of which the former seemed more significant statistically. The alpha cell activity had only one maximum peak at midafternoon and a minimum at 10 p.m. The beta-alpha cell nuclear cell ratio showed a minimum peak at 8 p.m. The possible causes of this rhythmic variation in islet cell function were discussed. S.B.B.

Hernberg, C. A.; Björkesten, G.; and Vannas, S. (Med. Dept. of the Maria Hosp., The Finnish Red Cross Hosp. & the Ophthal. Hosp. of Univ. of Helsinki, Finland): HYPOPHYSECTOMY IN THE TREATMENT OF RETINOPATHY AND NEPHROPATHY IN SEVERE JUVENILE DIABETES. Acta endocrinol. *31*:241-60, June 1959.

This is a report of ten patients with severe diabetic retinopathy and some, but not serious, diabetic nephropathy subjected to hypophysectomy mainly for the former condition. All but one lived beyond three months. The renal disorder did not progress, and in three patients proteinuria disappeared postoperatively. It was the opinion of the authors that the progression of the retinopathy had been retarded by the operation. The experimental nature of this operative approach to these diabetic complications was stressed by the authors. S.B.B.

Himwich, Williamina A.; and Sullivan, William T. (Galesburg State Res. Hosp., Galesburg, Ill.): CHEMICAL CONSTITUENTS OF BRAIN AS AFFECTED BY INSULIN HYPOGLYCEMIA, CONVULSIONS AND FEVER. J. Nerv. & Ment. Dis. *124*:21-26, July 1956.

The authors produced convulsions in rabbits and then measured the amount of sodium and potassium in the brain tissue. They found that with picrotoxin convulsions there was an elevation of blood potassium and an increase in sodium in the brain of the rabbits. Deep insulin hypoglycemia showed a decrease of potassium and an increase in the sodium in all brain areas. It was found with mild insulin reactions that the free glutamates rose and the combined values of glutamic acid fell and this was interpreted as suggesting that when there is deficiency of glucose to the brain tissue, glutamic acid may be utilized to maintain metabolism. R.C.C.

Ishihara, Ichiro; Komori, Yoshitaka; Saka, Yukio; and Suzuki, Kozo (Ishihara Lab. of the Research Inst. of Environmental Medicine, Nagoya University, Nagoya, Japan): UROPEPSIN AND URINARY ADRENOCORTICAL STEROIDS AFTER SUBCUTANEOUS ADMINISTRATION OF INSULIN. Ann. Rep. Research Inst. Environmental Med., Nagoya Univ., pp. 56-66, 1956.

Changes in circulating eosinophils, uropepsin, 17-hydroxycorticoids and 17-ketosteroids in urine were observed after subcutaneous injection of insulin in a dose of 0.15 units per kilogram of body weight in human subjects.

In the normal eosinopenic group, excretion of uropepsin was definitely elevated, while uropepsin was observed rather reduced in the group showing abnormal response of eosinophils. Statistically significant elevation of urinary total 17-hydroxycorticoids and a rise of 17-ketosteroids in the group with normal eosinopenic response as well as increased uropepsin indicate the adrenocortical activation.

It can be concluded that eosinopenic response and increased excretion of uropepsin after insulin stress suggest a possibility of the normal adrenocortical reserve. W.R.K.

Jaeger, D. A.; Drakontides, A. B.; Dimitrakis, M.; and Schneider, J. A. (Macrobiology Dept., Chas. Pfizer & Co., Brooklyn, N.Y.): ANTAGONISM OF SUCCINYLCHOLINE TO CHLORPROPAMIDE INDUCED HYPOGLYCEMIA. Proc. Soc. Exper. Biol. & Med. *101*:532-35, July 1959.

These authors present the results of experiments in cats and rats in which a reversal of induced hypoglycemia by means of chlorpropamide was obtained with succinylcholine. This hypoglycemic reversal with succinylcholine could not be obtained in the adrenalectomized animal. Experiments performed with ether, reserpine, and epinephrine further supported the view that this antagonism was mediated through the adrenal medulla. G.J.H.

Janes, Ralph G. (State University of Iowa, Coll. of Med., Iowa City, Ia.): THE RELATIONSHIP BETWEEN EYE AND KIDNEY PATHOLOGY IN THE DIABETIC RAT. A.M.A. Arch. Path. *67*:386-94, April 1959.

Alloxan-diabetic rats were studied with particular attention to ocular and renal pathology over periods of one week to eighteen months. Cataracts developed within three months but retinopathy was rare (one instance of a striate hemorrhage and two of aberrant retinal vessels). No retinal microaneurysms were seen. Hemorrhage into the anterior chamber of the eye was not infrequent and sometimes severe.

Renal changes consisting of glomerular capillary wall thickening and capillary dilatation were seen after three months of diabetes. Advanced renal changes consisting of glomerular sclerosis, glomerular nodular lesions, and tubular degeneration were concomitant with bleeding into the anterior chamber of the eye.

The major lesions seen in these diabetic animals were vascular in origin. The ocular lesions did not resemble those seen in the diabetic patient while those in the glomerulus did. H.L.W.

Kadota, Ichiro; and Kawachi, Yoshimi (Dept. of Med., Nat'l. Railway Hosp., Osaka, Japan): DIABETOGENIC ACTION OF ANALOGUES OF 8-HYDROXYQUINOLINE. Proc. Soc. Exper. Biol. & Med. *101*:365-66, June 1959.

As previously reported, some chelating agents for heavy metals have been shown to be diabetogenic in experimental animals. The authors tried a number of derivatives of 8-hydroxyquinoline in rabbits and demonstrated that hyperglycemia due to destructive changes of the pancreatic islet cells could be caused by 6-hydroxy-m-phenanthroline and 1-hydroxyacridine as well as 5-hydroxybenzo-f-quinoline. However, three derivatives of pteridine, picolinic acid, and dipicolinic acid failed to produce significant changes in blood sugar. G.J.H.

Kornerup, Tore; and Ström, Lors (Ophthalmic & Pediatric Clinics, Karolinska Sjukhuset, Stockholm): VITAMIN B_{12} AND RETINOPATHY IN JUVENILE DIABETES. Acta paediat. *47*:646-51, November 1958.

One hundred micrograms daily of B_{12} was given to a group of fifteen patients with diabetes and an age at onset of less than fifteen years and with an average duration of diabetes of 11.8 years. All showed signs of retinopathy. The controls were twenty-two juvenile diabetics without retinopathy, average duration of disease 8.5 years. The patients were followed for two years. After one year of B_{12} treatment, seven of the fifteen treated cases no longer had retinopathy, and this number increased in two years to eight. Only one of a group of twenty-two adult diabetics treated with B_{12} (duration of diabetes fifteen to twenty-eight years), onset before age fifteen years, showed a similar regression. The author believes that B_{12} may delay the development of incipient retinopathy in the juvenile diabetic. R.L.J.

Krahl, M. E.; Tidball, M. E.; and Bregman, E. (Dept. of Physiology, Univ. of Chicago, Ill.): PREPARATION AND ANTI-INSULIN ACTIVITY OF LIPOPROTEIN FRACTIONS FROM RAT SERUM. Proc. Soc. Exper. Biol. & Med. *101*:1-6, May 1959.

Procedures for routine preparation from the serum of both normal and diabetic rats of fractions uniformly inhibitory to the glucose uptake of rat diaphragms have been presented. The inhibitory activity of fractions from normal serum were enhanced by prior treatment of the animal with pituitary growth hormone whereas ACTH and TSH were inactive in this respect. The inhibitory fractions had the properties of β-lipoproteins and could be prepared free of lipids having electrophoretic mobility of albumin or α-globulins. The inhibitory fractions interfered with the stimulatory action of insulin on diaphragm muscle. The fraction from 1 ml. of diabetic or normal rat serum nullified the stimulatory effect of at least .0004-.001 units of insulin. G.J.H.

Lazarus, Sydney S.; and Volk, Bruno W. (Isaac Albert Res. Inst. of Jewish Chronic Hosp., Brooklyn, & Dept. of Pathol. of Albert Einstein Coll. of Med. of Yeshiva Univ., New York, N.Y.): PANCREATIC ADAPTATION TO DIABETOGENIC HORMONES. A.M.A. Arch. Path. *67*:456-67, April 1959.

Normal rabbits treated with cortisone over a period of weeks show an initial hyperglycemia which gradually declines with the passage of time. Similar findings are noted on treatment with cortisone plus glucagon though the early hyperglycemia is more marked than with cortisone alone.

Histological changes accompanying the amelioration of metadiabetes were degranulation and increased mitotic activity of pancreatic beta cells. New beta cells developed from nonvacuolated hyperplastic ductules.

Mongrel dogs subjected to partial pancreatectomy (50 per cent) were given growth hormone and maintained on a high carbohydrate diet. A persistent hyperglycemia was produced.

Progressive beta cell degranulation, ductular and beta cell glycogen infiltration occurred. Finally intra-insular cells ballooned out and their nuclei became pyknotic. No new formation of beta cells was noted.

This study details the histologic changes in the pancreas accompanying on the one hand, an adaptation of the animal to diabetogenic hormones and on the other, a failure of the animal to adapt with the supervention of permanent diabetes. H.L.W.

Lendle, L. (Inst. of Pharmacol., Univ. of Göttingen, Göttingen, Germany): QUESTION AND ANSWER. CARBON MONOXIDE POISONING AND DIABETES. German M. Month. *4*:25, January 1959.

A fifty-five-year-old woman was first found to have glycosuria one month after acute carbon monoxide poisoning. Can diabetes be regarded as a possible sequel of this form of poisoning, even if it occurred after one month? As a rule, so-called carbon monoxide diabetes, known since the days of Claude Bernard, occurs a few hours after the intoxication, subsiding after several hours or days. This disorder of the sugar metabolism, often diagnosed only by a glucose tolerance test, is no longer attributed to the temporary oxygen deficiency but to a direct disturbance in the diencephalon. There has been only one report on

diabetes as a late complication of CO poisoning (Lenin, L.: "Die Kohlenoxydvergiftung," Berlin 1920, p. 211): A builder suffered CO poisoning while working in a chimney; he was unconscious for three-quarters of an hour and for three days following there was no sign of glycosuria. As late as three months after the event he was found to have diabetes (6 per cent sugar in the urine). There were no other obvious causes for the condition. Provided there is no reason to suspect any other metabolic disturbances (any hereditary disease?) one may assume that some causal relationship exists between the carbon monoxide poisoning and the diabetes. W.R.K.

Leonards, Jack R.; and Martin, F. I. R. (Western Reserve Univ. Sch. of Med., Cleveland, Ohio, and Univ. Hospital, Cleveland, Ohio): INSULIN SENSITIVITY: A VARIANT OF INSULIN RESISTANCE. New England J. Med. *261*:68-70, July 9, 1959.

The present report describes a patient with moderate insulin resistance in whom a high concentration of insulin-like activity was demonstrated in the peripheral blood. This patient's insulin resistance was of a relatively mild degree (ca. 200 units), lasted for several weeks, and then terminated abruptly. During this period a large amount of insulin-like activity was demonstrated in the serum by two separate methods. Recent studies in this laboratory raise a serious question of the specificity of the rat epididymal fat pad for insulin assay of serum. However, in the present case, the great activity of the serum even at very high dilution suggests that the effect is due to exogenous insulin. The development of insulin resistance shortly after a cerebral thrombosis is of interest since, in laboratory animals, damage to the hypothalamus or brain stem can result in an elevated blood sugar. Furthermore, there is a clinical association between cerebral damage and hyperglycemia, and there have been reports of diabetes that apparently developed in association with hypothalamic tumors. However, no evidence could be found that these circumstances were associated with any significant insulin resistance, and it is pertinent that, in the present case, the resistance persisted for three weeks after the cerebral thrombosis whereas the recorded cases of hyperglycemia from cerebrovascular damage have lasted only a few days. W.R.K.

Ludin, Hans; and Asch, Thane (Dept. Radiology, The N. Y. Hosp.-Cornell Med. Center, New York, N.Y.): THE NORMAL LATERAL PNEUMOTOMOGRAPHIC APPEARANCE OF THE PANCREAS. Radiology 72:197-204, February 1959.

Pneumoperitoneum and retroperitoneal insufflation were carried out on twenty-six subjects by using nitrous oxide as a contrast agent instead of air or oxygen to lessen the possibility of air embolism. Tomography at different planes followed. The normal pancreas appears in cross section as a triangle with straight walls. A real bulging of the normally straight walls indicates an expansive process within the gland. The procedure is considered without risk and the radiation hazard unimportant. Limitations to the study are the unsatisfactory distribution of the injected gas, and adequate filling of the omental bursa does not invariably occur. The difficulty is greater in obese patients. Any acute or chronic inflammatory conditions can interfere with the perfusion of gas after injection. C.A.V.

Marble, Alexander (Asst. Clinical Professor of Medicine, Harvard Med. School; Physician Joslin Clinic and New England Deaconess Hospital, Boston, Mass.): ORAL HYPOGLYCEMIC AGENTS IN THE MANAGEMENT OF DIABETES. M. Clin. North America *42*:1163-77, September 1958.

This article is an excellent resume of where we now stand in the use of the oral hypoglycemic agents. There is a brief discussion of all agents including tolbutamide, carbutamide, sulfaisopropyl-thiadiazol (RP 2254) and phenethylbiguanide (DBI). The author includes a November 1957 report from the Joslin Clinic of 772 patients treated with tolbutamide, of whom 407 or 52.6 per cent were felt to have good control of their hyperglycemia and 143, or 18 per cent were thought to have fair control. Chlorpropamide, according to studies, milligram for milligram, has a greater hypoglycemic effect than carbutamide and seems to be more slowly excreted from the body. The use of phenethylbiguanide (DBI) and a brief review of the literature concerning this drug are also discussed, not only in adults but in some children with diabetes. There appears to be a rather high incidence of anorexia, nausea and vomiting and diarrhea when doses of the drug are increased to therapeutic levels necessary to acquire the proper hypoglycemic effect. The mode of action of DBI is not known. R.C.C.

Mirsky, I. Arthur; Perisutti, Gladys; and Davis, Neil C. (Univ. Pittsburgh, Sch. of Med., Pittsburgh, Pa.): THE DESTRUCTION OF GLUCAGON BY THE BLOOD PLASMA FROM VARIOUS SPECIES. Endocrinology *64*:992-1001, June 1959.

The proteolytic enzyme, plasmin, found in human plasma, is responsible for the hydrolysis of glucagon. Plasmin is derived from an inactive euglobulin fraction termed plasminogen. Streptokinase enhances the conversion of plasminogen to plasmin in the presence of a "proactivator" found in human, monkey and cat plasmas. Proactivator is shown to be absent or very low in the plasmas of guinea pig, chicken, rabbit, cow and dog. A more general action of plasmin is suggested in the production of physiologically active polypeptides after exposure of the organism to noxious stimuli. H.L.W.

Mukherjee, B. (India): DIABETES MELLITUS: SCOPE OF THERAPEUTICS. Licentiate *8*:211-19, October 1958.

Folk medicines of several lands contain remedial agents of plant, animal or mineral origin which are alleged to have striking antidiabetic properties. Most of these substances exert their hypoglycemic actions through deleterious interferences with metabolic processes, often effective through hepatic or renal injury. In India the author reports a sudden burst of patriotism in the medical field. A large number of indigenous drugs have been tried in the treatment of diabetes mellitus. Some of these trials have often bordered on criminality, having been carried out even without previous toxicologic or pharmacologic studies. The author condemns the unethical nature of these trials. Diabetes is a disease in which therapy is well known. Therefore any attempt of a treatment of this disease with drugs whose actions and reactions are not known cannot be condoned by any right-thinking medical person. The possibility of finding an insulin substitute from plant or mineral kingdom is quite remote. The trials are designed only to find some hypoglycemic agents. Diabetes does not belong to that group of diseases where the finding of another hypoglycemic agent will be considered a great achievement. The new group of synthetic compounds which has come into the picture accomplishes this blood sugar lowering action quite satisfactorily. These compounds are relatively safe to use and have undergone large numbers of trials. Therefore no greater glory is to be obtained by merely finding a few more hypoglycemic agents from indigenous plants. W.R.K.

Nichols, Nancy; and Nichols, George Jr. (Baker Clin. Res. Lab., New England Deaconess Hosp., & Depts. of Med. & Biochem., Harvard Med. School, Boston, Mass.): THE EFFECT OF ALLOXAN DIABETES AND ACIDOSIS ON THE MINERAL AND WATER CONTENT OF BONE. J. Clin. Invest. 37:1676-85, December 1958.

Experiments were designed to examine effects of diabetes and of acidosis separately upon the bone storage depot of body sodium and calcium. Normal rats and alloxan diabetic rats with diabetes of varying duration were studied. Acidosis was produced by intraperitoneal injection of ammonium chloride. The decrease in *p*H produced was equal in both groups, while ketonemia was greater in the normals.

In the absence of acidosis, untreated alloxan diabetes did not differ from the controls in sodium and calcium losses from bone. With acidosis the sodium content decreased 12 per cent, and calcium 5 per cent. The physiological implications of these findings were discussed especially with respect to the calculation of total chloride space and water volume of bone. S.B.B.

Pedersen, Jørgen; and Nissen, Nis I. (Frederiksberg Hosp., Med. Dept. E, Copenhagen, Denmark): DIABETES WITHOUT OR WITH SLIGHT, INTERMITTENT GLUCOSURIA. Acta med. scandinav. *163*:477-88, May 9, 1959.

The present study revealed that asymptomatic diabetes without or with slight intermittent glucosuria is common among the older age groups. In order to be able to make a diagnosis, the disease must be borne in mind as a possible diagnosis, and blood sugar determination should be carried out instead of repeated investigation for glucosuria. It is not advisable to interpret the absence of glucosuria and a normal fasting blood sugar as reliable evidence against diabetes in patients who are over fifty years of age, especially if they are potential diabetics.

Determination of the twenty-four-hour blood sugar has proved of value in these cases and should be more widely used in hospitals. (The twenty-four-hour blood sugar is the average of the blood sugars taken at 8:30 a.m., 12 noon, 3 p.m. and 8 p.m. for three or more days with the afebrile patient on a full diet.)

The threshold problem deserves renewed investigations, especially in potential diabetics, since hyperglycemics with a high renal threshold probably make up a special category. B.F.K.

Pomeranze, Julius; Feldman, Harold; King, Edward J.; Lucariello, Ralph J.; and Simon, Julius (New York, N.Y.): A CORRELATION OF SERUM LIPIDS, LIPOPROTEINS, AND BLOOD SUGAR LEVELS IN DIABETES MELLITUS. Circulation *18*:508-09, September 1958. (Paper #70 presented at the Twelfth Annual Meeting of the American Society for the Study of Arteriosclerosis, San Francisco, Oct. 24, 1958.)

The authors studied 104 patients of all ages with diabetes of varying degrees of severity for serum lipid, electrophoretic lipoprotein analysis and blood sugar level determinations. Some patients whose diabetes was very poorly controlled were then adequately controlled and comparison studies were made. Three final results were obtained. The beta-lipoprotein, cholesterol and total lipid levels were significantly higher in the older diabetic patient, despite the increased severity of the disease in younger patients. There appears to be some degree of correlation between elevated blood sugar levels and beta-lipoprotein and total cholesterol levels. Improvement in diabetic control, within certain limits, creates more normal levels of total lipids, cholesterol and beta-lipoproteins. R.C.C.

Rodríguez, Ricardo R. (Inst. of Biology & Experimental Medicine, Buenos Aires, Argentina): THE KIDNEY IN DIABETES MELLITUS. Rev. Asoc. méd. argent. 70:118, May 15-30, 1956.

Prevention of diabetic nephropathy in diabetic rats as a result of alloxan administration and subtotal pancreatectomy was achieved by the administration of insulin. Glomerular and tubular lesions were not present in these animals unlike the untreated diabetic rats that developed these typical kidney lesions. R.D.A.

Rossi, E. (Univ. Paediat. Clin., Berne, Switzerland): GLUCAGON. Triangle *4*:13-23, April 1959.

The biochemistry of glucagon has been largely elucidated, whereas its physiologic function is for the most part still obscure. Glucagon is a polypeptide, the structure of which is known. Chemical and biological methods for its identification, isolation and preparation are now available. In very small doses, it exerts an influence on a complex enzyme system in the liver which controls the dynamic equilibrium between active and inactive phosphorylase. The liver possesses an enzyme system which inactivates glucagon. The hormone character of glucagon is no longer contested by the majority of authors. It is highly probable that glucagon is secreted by the α cells of the islets of Langerhans and certain analogous cells of the gastrointestinal tract. It integrates the regulation of carbohydrate metabolism, and, in our opinion, acts as a synergist of insulin, displacing glucose from the liver into the periphery and enhancing its utilization. In contrast, many authors regard glucagon as an antagonist of insulin, believing that it has a diabetogenic action and participates in metabolic regulation alongside the hormones of the anterior lobe of the pituitary, the glucocorticoids, the androgens, and the sympathetic nervous system. Further work is needed to clarify these hypotheses. W.R.K.

Schumacher, O. Peter; Fee, Beverly A.; McCullagh, E. Perry; and Kovacs, Leslie L. (Cleveland Clinic, Cleveland, Ohio): CHLORPROPAMIDE, A NEW DRUG FOR DIABETES MELLITUS: CLINICAL STUDIES. A PRELIMINARY REPORT. Cleveland Clin. Quart. 26:12-14, January 1959.

Fifty-three patients with diabetes mellitus were treated with chlorpropamide for one to seven months. The drug therapy was effective in twenty-eight of thirty-one adult patients with stable diabetes. It was effective in a number of these patients in whom diabetes was not well controlled with tolbutamide. Of four adult patients with unstable diabetes, one patient obtained a decrease in insulin requirement; the other three patients were unaffected by chlorpropamide therapy. With this drug temporary control was obtained in six of eighteen young diabetic patients, and normal glycemia has been maintained in one of them without insulin administration. Severe side effects occurred only in the two patients who required more than 1 gm. of chlorpropamide daily. B.F.K.

Sellman, J. C.; Perkoff, G. T.; Null, F. C.; Kimmel, J. R.; and Tyler, F. H. (Univ. of Utah Coll. of Med., Salt Lake City, Utah): HYPOGLYCEMIA ASSOCIATED WITH MASSIVE INTRA-ABDOMINAL MESOTHELIAL-CELL SARCOMA. New England J. Med. 260:847-52, April 23, 1959.

A case of massive intra-abdominal mesothelial-cell sarcoma with associated hypoglycemia is reported. Insulin-like activity could not be demonstrated in extracts from the tumor. The tumor was found to have high levels of amino acids and glycogen. The high glycogen and amino acid levels, together

with the absence of demonstrable evidence of other mechanisms for the production of hypoglycemia, suggest that excessive glucose consumption by the tumor was responsible for the hypoglycemia in this patient. W.R.K.

Skillern, Penn G. (Dept. of Endocrinol., The Cleveland Clinic Foundation, and The Frank E. Bunts Educational Inst., Cleveland, Ohio): CLINICAL ASPECTS OF HYPOGLYCEMIA. M. Rec. & Ann. *51*:793-801, September 1958.

A practical clinical classification of spontaneous hypoglycemia, which includes both organic and functional causes, is presented:

Classification of Spontaneous Hypoglycemia

I. Organic
  A. Hyperinsulinism
    1. Endogenous
      a. Pancreatic islet-cell adenoma
        (1) Single
        (2) Multiple
        (3) Diffuse adenomatosis
        (4) Aberrant
      b. Pancreatic islet-cell carcinoma
        (1) Localized
        (2) With metastasis
    2. Exogenous
      a. Treatment of diabetes mellitus
      b. Factitious (surreptitious) administration of insulin
  B. Associated with spindle-cell fibrogenic tumor
  C. Hepatic disease
  D. Anterior pituitary failure
  E. Adrenocortical failure
  F. Familial idiopathic hypoglycemia in children
  G. Galactosemia
II. Nonorganic
  A. Functional hypoglycemia
  B. Functional hypoglycemia secondary to diabetes mellitus
  C. Functional hypoglycemia following gastric surgery.

W.R.K.

Sloper, J. C. (London, England): DIABETES INSIPIDUS: THE CONTRIBUTION OF HISTOCHEMICAL AND ISOTOPE TECHNIQUES TO THE INVESTIGATION OF ITS PATHOGENESIS. Proc. Roy. Soc. Med. *51*:335-39, May 1958.

Recent investigations into the pathogenesis of diabetes insipidus are reviewed. These have been based on the hypothesis, still disputed, that antidiuretic hormone is formed by certain secretory neurons of the hypothalamo-neurohypophyseal system rather than by "pituicytes." It follows from this hypothesis that the essential abnormality in diabetes insipidus is the destruction or dysfunction of these neurons, and discussion relates to their reactions to stress and to injury. An outline of certain newer histochemical technics to the investigation of these neurons is given, both for experimental animals and man. G.A.W.

Sokal, Joseph E.; and Gerszi, Kornell E. (Div. of Med., Roswell Park Memorial Inst., Buffalo, N.Y.): HUMAN LIVER GLYCOGEN LEVELS. J. Lab & Clin. Med. *53*:876-81, June 1959.

The authors present the results of determination of human liver glycogen from liver biopsies obtained at surgery. The mean value for thirty patients without jaundice was 2.2 per cent and did not differ significantly from the mean for six jaundice patients, which was 2.12 per cent. In reviewing the individual determinations, the authors could find no correlation between liver glycogen values and diagnosis, nutritional status, or age. An additional case of excessive storage of glycogen in a patient with diabetes is reported in whom the value for liver glycogen was 12.9 per cent. The data presented agree with other operative series reported and with results obtained from needle aspiration biopsy in unanesthetized patients. Review of the combined data indicates that the upper limit of the normal range for human liver glycogen after an overnight fast is about 6 per cent. G.J.H.

Steiness, Ib (I and II Med. University Depts., Aarhus Kommune-hospital, Aarhus, Denmark): VIBRATORY PERCEPTION IN DIABETICS: A BIOTHESIOMETRIC STUDY. Acta med. Scandinav. *158*:327-35, Sept. 20, 1957.

Biothesiometric measurements of the vibratory perception were performed in sixty-three diabetic patients under standardized conditions. No significant difference was revealed between the two sides of the body. The threshold of the legs was higher than that of the arms; in both, it increased significantly with age, most markedly in the legs. The threshold of vibratory perception of both the upper and lower limbs is significantly increased in diabetics, but there is no significant difference between the slopes of the regression lines with regard to age at the corresponding sites of measurement in diabetics and normal subjects. As distinct from normal individuals, diabetics show no difference between the two sexes in the vibratory perception in the lower limbs. This may suggest that the impairment of vibratory perception is due to vascular disease consequent to nerve damage caused by diabetic angiopathy. A significant positive correlation between the increased threshold and the duration of diabetes was demonstrated in the lower limbs, but a similar correlation could not be demonstrated in the upper limbs. A positive correlation was revealed between the increased threshold of vibratory perception and other signs of neuropathy; but no correlation with the severity of diabetes as expressed by the insulin requirement, with symptoms of neuropathy or with the estimated regulation could be demonstrated. B.F.K.

Unger, Roger H.; Madison, Leonard L.; and Amil, Manuel (Dept. of Med., The Univ. of Texas Southwestern Med. Sch., the Parkland Memorial Hosp., Metabolic Clinic, and the Dept. of Med., V. A. Hosp., Dallas, Tex.): THE APPARENT SUPERIORITY OF TOLBUTAMIDE OVER INSULIN IN THE REGULATION OF SELECTED CLINIC PATIENTS. South. M. J. *51*: 1603-10, December 1958.

Under the difficult conditions which prevail in the charity diabetes clinic, tolbutamide therapy was instituted in a hundred selected patients, ninety-six of whom had been receiving insulin. Seventy-two of these subjects have been successfully controlled with the drug for periods of from four to seven months. Seventy-eight per cent of these have had mean fasting blood sugar levels of 150 mg. or less, and 47 per cent have averaged below 125 mg. per 100 cc., a record of smooth and effective control surpassing by a wide margin that of prior insulin regulation in these same subjects. The apparent superiority of tolbutamide therapy over insulin in certain diabetics is critically analyzed, in view of certain uncontrolled variables in the two therapeutic regimens being compared. It is

concluded that, in at least some diabetics, the apparently greater effectiveness of oral therapy is genuine. The major hazard of tolbutamide therapy is diabetic acidosis resulting from the termination of insulin therapy in patients dependent upon exogenous insulin. This danger can be minimized by daily observation of patients during the conversion period or by a program of gradual insulin withdrawal as recommended by others. Tolbutamide has thus far proved to be an effective therapeutic agent which has simplified the management of clinic diabetics. W.R.K.

Wagner, Martin J.; and Muhler, Joseph C. (Baylor University College of Dentistry, Dallas, Tex., Indiana University Medical Center, Indianapolis, Ind.): RETENTION OF AQUEOUS FLUORIDE BY ALLOXAN-DIABETIC RATS. J. Am. Dent. A. *59*:81-83, July 1959.

Groups of alloxan-diabetic and normal rats received fluoridated drinking water ad libitum for forty days. All were sacrificed and the carcasses analyzed for fluoride. The fluoride ingestion and excretion were similar in both groups . W.R.K.

Walker, Robert S. (Law Hosp., Carluke, Lanarkshire, Scotland): THE LONG-TERM USE OF CARBUTAMIDE (BZ-55) IN THE TREATMENT OF DIABETES MELLITUS. Acta endocrinol. *31*:261-66, June 1959.

This is a review of experience with carbutamide treatment of thirty-nine mild, older and twenty-six younger, more acute diabetic patients. Most were treated with low doses: 1.5 gm. initially, down to 0.5 gm. every other day, where possible, later.

Progressive increase in activity of the drug with time was noted in the first group. The remission of the diabetes persisted less than three months after withdrawal of the drug. In both groups, return to insulin therapy was sometimes accompanied by an increase in insulin needs. Of the younger group of twenty-one patients, three of five stable juvenile diabetics could be treated by carbutamide alone, eleven of the twenty-one patients could reduce insulin needs with use of supplements of carbutamide, and two of these twenty-one showed a stabilizing effect on the diabetes of such oral supplements. Two of the forty-nine patients showed serious hematological or hepatic complications but these were patients on larger doses of the drug.

The author feels that since this drug is effective at very low dosage levels, its continued use can be justified since toxicity is lowered sufficiently at such low levels. S.B.B.

Weaver, Neill K.; and Perret, James T. (Baton Rouge Refinery, Baton Rouge, La.): THE DIABETIC IN INDUSTRY. M. Bull. *18*:304-16, November 1958.

Ninety diabetics were present in a large refinery and petrochemical plant employing 7,140 workers. Clinically, their disease was characterized by a relative lack of complications, although degenerative conditions may predispose the older patient-employees to work restrictions and disability retirement. Sickness absenteeism of the diabetic subjects was not significantly elevated when compared to that of nondiabetic employees. The diabetic employees have performed effectively for 774 man-years in all job classifications of the refinery. A.S.R.

White, Thomas T.; and Magee, D. F. (Depts. of Surg. and Pharmacology, Univ. of Washington, Sch. of Med., Seattle, Wash.): ATTEMPTED OPACIFICATION OF THE PANCREAS WITH THE USE OF IODINATED DYES. Radiology *72*:238-41, February 1959.

Rabbits and dogs were injected intravenously with aniline and iodinated dyes. Some of these were recovered in the pancreatic secretions of animals with fistulas. However, the opacity noted in intact animals was found to be in the intestine or the stomach. The attempt to opacification was not successful. C.A.V.

Williams, R. D. Brooke (Dept. of Pediatrics, University of Rochester, Rochester, N.Y.): A DISTURBANCE OF CARBOHYDRATE METABOLISM CAUSED BY LESIONS OF THE CENTRAL NERVOUS SYSTEM: REPORT OF A CASE IN A CHILD WITH A HYPOTHALAMIC TUMOR AND REVIEW OF THE CLINICAL REPORTS IN THE LITERATURE. J. Pediat. *54*:299-312, March 1958.

The author gives an extensive review of cases reported in the literature with diabetes associated with hypothalamic damage, and feels that there is some clinical evidence to support the experimental work, which is also reviewed in the article, that transient hyperglycemia and glycosuria can be produced by lesions or stimulation of the hypothalamic region. He reports the case of a four-year-old child with acute onset of stiff neck, high temperature, convulsive twitchings on easy stimulation as well as a 4-plus urine sugar and hyperglycemia (no initial value was stated). At autopsy a tumor, an invasive ependymoma involving the base of the brain infiltrating the subthalamic region, both thalami, and the hypothalamus, was found. He suggests that detailed examinations of the brain and hypothalamus, when possible, be made in every case of diabetes with cerebral symptoms to help elucidate the problem. R.L.J.

Wright, Paul A. (Dept. of Zoology, Univ. of Michigan, Ann Arbor, Mich.; & Marine Biological Lab., Woods Hole, Mass.): BLOOD SUGAR STUDIES IN THE BULLFROG, RANA CATESBIANA. Endocrinology *64*:551-58, April 1959.

The problem of low or absent levels of glucose in the blood of the bullfrog is investigated. The nutritional state of the animal plays no significant role. Blood loss, intra-arterial glucagon, epinephrine, and extracts (presumed to contain glucagon) of frog and fish gastrointestinal tract all produced a rise in blood sugar. Alloxan, beef STH, and bullfrog pituitary extract did not. The production of and tolerance for a low or absent blood sugar are discussed. R.L.J.

Yang, Yong H.; and Hunter, Warren C. (Dept. of Path., Univ. of Oregon Med. Sch., Portland, Oregon): THE RELATION OF THE PANCREATIC DUCTS TO THE ISLETS OF LANGERHANS. A.M.A. Arch. Path. *67*:505-14, May 1959.

The origin of pancreatic islet tissue from ductules and/or acinae is discussed and the literature reviewed. Three cases are presented in which duct tissue is shown to proliferate and form islet cells. H.L.W.

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Eighth Postgraduate Course in Diabetes and Basic Metabolic Problems

Los Angeles, California, Jan. 20-22, 1960

Preliminary Program

The American Diabetes Association will hold its Eighth Postgraduate Course in Diabetes and Basic Metabolic Problems in Los Angeles, California, Jan. 20, 21 and 22, 1960. Open to Doctors of Medicine, the Course was developed by the Committee on Professional Education of the American Diabetes Association.

Thaddeus S. Danowski, M.D., Pittsburgh, is Chairman of the Committee and Director of the Course. Arthur R. Colwell, Sr., M.D., Chicago, and Jerome W. Conn, M.D., Ann Arbor, are Vice Chairmen of the Committee. The Course will be given in cooperation with the Schools of Medicine of The College of Medical Evangelists, University of California at Los Angeles and the University of Southern California.

Roy F. Perkins, M.D., the Association's Governor for Southern California, is Chairman of the Local Committee whose members include: Representing the School of Medicine, The College of Medical Evangelists—*William W. H. Pote, Jr., M.D., and Roy F. Perkins, M.D.;* representing the School of Medicine, University of California at Los Angeles—*David H. Solomon, M.D., and Samuel Soskin, M.D.;* representing the School of Medicine, University of Southern California—*Helen E. Martin, M.D.*

REGISTRATION: A Preliminary Program which includes an application form is being mailed to members of the Association and subscribers of the Journal. This form should be filled out and mailed together with the fee to the American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y. Applications will be accepted in the order received and registrations officially confirmed. The registration desk will be open from 3:00 p.m. to 9:00 p.m. on Tuesday, January 19, and will open again at 8:00 a.m. on Wednesday morning, January 20.

FEES: The registration fee for the three-day Course is $40 for members of the American Diabetes Association, and $75 for nonmembers. The full fee must be paid at the time of filing application and includes the cost of the Banquet to be held Wednesday evening, January 20. The fee will be returnable by the Association to any registrant who submits his withdrawal in writing not later than January 8.

Fellows and residents engaged in full-time study in medicine and allied sciences in schools and hospitals in the Los Angeles area may attend the scientific sessions at a nominal fee of $10; interns may attend without charge. Fellows, residents and interns should register by January 8. Medical students in the Los Angeles area may register without charge after January 8, if space permits.

HOTEL ACCOMMODATIONS: The Ambassador will serve as the headquarters hotel. Reservation cards will be sent to registrants.

POSTGRADUATE CREDIT: The American Academy of General Practice will give eighteen hours' Postgraduate Credit for the Course (Category II).

THE FOLLOWING TOPICS AND DISCUSSIONS (SUBJECT TO CHANGE) WILL BE COVERED DURING THE 3-DAY COURSE.

WEDNESDAY MORNING, JANUARY 20

9:00 Welcome by T. S. Danowski, Director; Roy F. Perkins, Chairman, Local Committee; Francis D. W. Lukens, President

I. FUNDAMENTALS OF DIABETES

9:10 Normal Metabolic Patterns and Interrelationships—Sidney Roberts
9:55 Therapeutic Implications, Fundamentals of Diabetes and Oral Hypoglycemic Agents—Samuel Soskin
10:25 *Intermission*
10:40 Pathologic Anatomy of Diabetes—Arnold Lazarow
11:25 Disturbances of Normal Physiology in the Diabetic State—Francis D. W. Lukens

WEDNESDAY AFTERNOON, JANUARY 20

FUNDAMENTALS OF DIABETES (Continued)

Actions and Use of the Hypoglycemic Agents

2:00 Insulin—Rachmiel Levine
2:30 Mechanism of Action of the Hypoglycemic Agents: Present Status—Albert E. Renold
3:00 Clinical Use of Oral Compounds—Alexander Marble
3:30 *Intermission*

3:45 to 5:00 *Panel*: Practical Applications of Basic Mechanisms in the Diabetic State—Rachmiel Levine, Moderator; Samuel Soskin, Francis D. W. Lukens, Albert E. Renold, Alexander Marble

WEDNESDAY EVENING, JANUARY 20

6:30 Social Hour (by subscription)
7:30 Banquet

THURSDAY MORNING, JANUARY 21

II. DIAGNOSTIC AND THERAPEUTIC CONSIDERATIONS

9:00 The Potential Diabetic—Arthur R. Colwell, Sr.
9:25 The Juvenile or Unstable Diabetic—T. S. Danowski
9:50 The Maturity-Onset (Stable) Diabetic—Alexander Marble
10:15 The Diabetic Under Stress—Peter H. Forsham
10:30 *Intermission*
10:50 to 12:00 *Round Table Conferences*: Diagnostic and Therapeutic Considerations

THURSDAY AFTERNOON, JANUARY 21

III. CLINICAL SESSIONS IN DIABETES, OR DIABETES IN REVIEW

2:00 Skin Manifestations of Diabetes—Molleurus Couperus
2:10 Unusual Biochemical Manifestations of Diabetic Acidosis—Helen E. Martin
2:25 Secondary Diabetes—Josiah Brown
2:40 Renal Problems (Pyelonephritis, Kimmelstiel-Wilson Syndrome)—Hugh A. Edmondson
3:00 Neurological Changes in Diabetes (Peripheral Neuritis, Neurogenic Bladder, Visceral Neuropathy, and Neuropathic Joints)—Kendrick Smith
3:15 Eye Changes in Diabetes (Cataracts and Retinitis)—Dale L. Hauck
3:30 Hypoglycemia (to Insulin and Oral Agents)—Burt Cochran, Jr.
3:45 *Intermission*
4:00 to 5:00 *Round Table Conferences*: Clinical Sessions in Diabetes, or Diabetes in Review

FRIDAY MORNING, JANUARY 22

IV. VASCULAR COMPLICATIONS OF DIABETES

9:00 Vascular Changes as Observed by Electron Microscopy—J. Francis Hartmann
9:20 Dietary Factors in Vascular Disease—Laurance W. Kinsell
9:40 Coronary Artery Heart Disease in the Diabetic—George C. Griffith
10:00 Peripheral Vascular Surgery in the Diabetic—Frank C. Wheelock, Jr.
10:20 Vascular Changes in Diabetics of Twenty or More Years' Duration—Eugene E. Berman
10:40 *Intermission*
10:55 to 12:25 *Panel*: Vascular Problems in Diabetes—Arthur R. Colwell, Sr., Moderator; Arnold Lazarow, Laurance W. Kinsell, George C. Griffith, Frank C. Wheelock, Jr.

FRIDAY AFTERNOON, JANUARY 22

V. DIABETES ON THE RESEARCH FRONTIER

Relative Importance of the Liver and Extrahepatic Tissues in the Metabolism of Glucose

1:35 Liver—Cross-circulation Studies—Piero P. Foa
2:05 Intermediary Metabolism of Extrahepatic Tissues—Arne N. Wick

Special Phases of Insulin Action

2:35 Insulin Antibodies—Solomon A. Berson
2:55 Insulin Antagonists—James B. Field
3:15 *Intermission*
3:30 Bio-assay and Summation of Insulin Conference—Peter H. Forsham
3:50 Comments and Summary—A. Baird Hastings, Douglas R. Drury
4:10 Newer Diagnostic Methods in Diabetes—Jerome W. Conn
4:40 Current Research—Charles H. Best

FACULTY OF THE EIGHTH POSTGRADUATE COURSE

EUGENE E. BERMAN, M.D., *Instructor in Medicine, University of Southern California; Attending Physician, Los Angeles County General Hospital; Cedars of Lebanon Hospital, Los Angeles, California.*

SOLOMON A. BERSON, M.D., *Chief, Radioisotope Service, Veterans Administration Hospital, Bronx, New York.*

CHARLES H. BEST, C.B.E., M.D., F.R.S., *Professor of Physiology; Director and Professor, Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada.*

JOSIAH BROWN, M.D., *Assistant Professor of Medicine (in residence), University of California at Los Angeles, Los Angeles, California.*

BURT COCHRAN, JR., M.D., *Assistant Clinical Professor of Medicine, Department of Medicine, The College of Medical Evangelists; Attending Physician, The College of Medical Evangelists Diabetes Service, Los Angeles County General Hospital, and Diabetes Training Center, White Memorial Hospital, Los Angeles, California.*

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JEROME W. CONN, M.D., *Professor of Medicine and Director, Metabolism and Research Unit, University of Michigan Medical School; Chief, Department of Endocrinology and Metabolism, University Hospital, Ann Arbor, Michigan.*

MOLLEURUS COUPERUS, M.D., *Associate Professor of Clinical Dermatology, The College of Medical Evangelists; Chairman, Department of Dermatology, White Memorial Hospital; Staff Physician, Los Angeles County General Hospital and Good Samaritan Hospital, Los Angeles, California.*

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DOUGLAS R. DRURY, M.D., *Professor and Chairman, Department of Physiology, University of Southern California, Los Angeles, California.*

HUGH A. EDMONDSON, M.D., *Professor and Head, Department of Pathology, University of Southern California, Los Angeles, California.*

JAMES B. FIELD, M.D., *Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.*

PIERO P. FOA, M.D., *Professor of Physiology and Pharmacology, and Associate in Medicine, The Chicago Medical School; Associate, Metabolic Clinic, Mt. Sinai Hospital, Chicago, Illinois.*

PETER H. FORSHAM, M.D., M.A., *Professor of Medicine and Pediatrics, Chief of Endocrinology and Metabolism, Director of Metabolic Research Unit, University of California, School of Medicine; Active Physician, University of California Hospitals, San Francisco, California.*

GEORGE C. GRIFFITH, M.D., *Professor of Medicine (Cardiology), University of Southern California, Los Angeles, California.*

J. FRANCIS HARTMANN, PH.D., *Professor of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota.*

A. BAIRD HASTINGS, PH.D., *Member, Scripps Clinic and Research Foundation, La Jolla, California.*

DALE L. HAUCK, M.D., *Assistant Clinical Professor of Ophthalmology, The College of Medical Evangelists; Senior Attending Physician, Department of Ophthalmology, White Memorial Hospital, Los Angeles, California.*

LAURANCE W. KINSELL, M.D., *Director, Institute for Metabolic Research, Alameda County Medical Institutions, Oakland, California.*

ARNOLD LAZAROW, M.D., PH.D., *Professor and Head, Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota.*

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ALEXANDER MARBLE, M.D., *Assistant Clinical Professor of Medicine, Harvard Medical School; Joslin Clinic and New England Deaconess Hospital, Boston, Massachusetts.*

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ALBERT E. RENOLD, M.D., *Assistant Professor of Medicine, Harvard Medical School; Director, Baker Clinic Research Laboratory, Boston, Massachusetts.*

SIDNEY ROBERTS, PH.D., *Professor of Physiological Chemistry, University of California at Los Angeles; Consultant in Metabolism, Veterans Administration Hospital, Los Angeles, California.*

KENDRICK SMITH, M.D., *Associate Professor of Clinical Medicine, University of Southern California; Chief of Diabetic Service, Los Angeles County General Hospital, Los Angeles, California.*

SAMUEL SOSKIN, M.D., *Associate Clinical Professor of Medicine, University of California at Los Angeles; Attending Physician and Chief of Diabetic Service, Cedars of Lebanon Hospital, Los Angeles, California.*

FRANK C. WHEELOCK, JR., M.D., *Instructor in Surgery, Harvard Medical School; Assistant Surgeon, Massachusetts General Hospital; Surgeon, New England Deaconess Hospital, Boston, Massachusetts.*

ARNE N. WICK, PH.D., *Associate Professor of Chemistry, San Diego State College, San Diego, California.*

20th ANNUAL MEETING

As previously announced, the Twentieth Annual Meeting of the American Diabetes Association will be held in Miami Beach, Florida, June 11-12, 1960, at the Deauville Hotel, prior to the annual session of the American Medical Association. Additional information about the Annual Meeting together with a hotel reservation card was mailed to members of the Association on November 6.

SCIENTIFIC PROGRAM

Physicians and other scientists who would like to present papers at the Scientific Sessions are invited to submit abstracts to Blair Holcomb, M.D., Chairman of the Committee on Scientific Programs. Seven copies of the abstracts, which are requested in order to expedite review by the Committee, should be sent as soon as possible, but not later than March 1, 1960, to Dr. Holcomb in care of the national office.

BOARD OF STATE GOVERNORS

New officers were elected to the Board of State Governors on June 5, 1959, at the time of the Nineteenth Annual Meeting. Joseph L. Izzo, M.D., Rochester, New York, was elected Chairman to succeed Christopher J. McLoughlin, M.D., Atlanta, Georgia. By reason of this office Dr. Izzo will serve as an ex officio member of the Council of the American Diabetes Association.

Joseph I. Goodman, M.D., Cleveland Heights, Ohio, was elected Vice Chairman, and Robert L. Reeves, M.D., Seattle, Washington, was re-elected Secretary of the Board.

Governors who will serve for the organizational year 1959-60 are:

Term Expiring June 1960

State	*Governor*
Alabama	Samuel Eichold
Arizona	Eleanor Waskow
Arkansas	Hal Dildy
California	
Northern & Nevada	Leo Goodman
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Term Expiring June 1961

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Louisiana	Daniel W. Hayes
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Nebraska	Morris Margolin
Nevada	See Northern California
New Hampshire	Jackson W. Wright
New Jersey	Benjamin Saslow
New York	
Eastern	Alfred E. Fischer
Western	Joseph L. Izzo
North Carolina	William M. Nicholson
North Dakota	Edgar A. Haunz

Term Expiring June 1962

Ohio	Cecil Striker
Oklahoma	Robert C. Lawson
Oregon	Wm. Richey Miller
Pennsylvania	
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Committees, 1959-60

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In addition, Chairmen of Committees on Employment of Affiliate Associations will serve by invitation.

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JANUARY 1 DEADLINE FOR 1960 LILLY AWARD

Nominations for the fourth Lilly Award must be submitted to the American Diabetes Association before Jan. 1, 1960. The annual award, which is supported by Eli Lilly and Company and consists of $1,000 and a medal, will be presented at the Twentieth Annual Meeting of the ADA June 11-12, 1960. The following stipulations govern the contest.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before January 1 of the year of the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to January 1 of the same year of the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

8th GRADUATE AND MEDICAL STUDENT-INTERN ESSAY CONTEST

The 1959-60 Graduate and Medical Student-Intern Essay Contest is open to graduate students, medical students, interns and physicians within two years after their graduation from medical school.

An award of $250 will be given to the author or authors of the best paper reporting original work, whether laboratory investigation or clinical observation. One hundred dollars ($100) will be awarded for the best review article or case report.

The papers will be judged on the basis of the value of the material and method of presentation. Any subject relating to diabetes and basic metabolic problems may be selected.

Members of the American Diabetes Association and subscribers to DIABETES are asked to encourage graduate students, medical students, interns and physicians within two years after their graduation from medical school to enter the contest.

Contestants should submit the original and two copies of their papers. Typewritten and double-spaced, all manuscripts should be mailed by April 1, 1960, to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y.

NEW MEMBERS

The following were elected as of Oct. 1, 1959:

Active

Indiana	
Ashmore, James	Indianapolis
Iowa	
Connor, William E.	Iowa City
New York	
McEvoy, Helen M.	Brooklyn
Volk, Bruno W.	Brooklyn
Ohio	
Schumacher, O. Peter	Cleveland
Pennsylvania	
Mills, Lewis C., Jr.	Philadelphia
Wolfe, Charles R.	Towanda
Texas	
Jacobs, Milton S.	San Antonio
Siperstein, Marvin D.	Dallas

NEWS OF AFFILIATE ASSOCIATIONS

The CONNECTICUT DIABETES ASSOCIATION will meet on Wednesday, November 18, in New Haven, during Diabetes Week. Francis D. W. Lukens, M.D., Philadelphia, will address the New Haven Medical Association at 8:15 p.m. on "Assessment of Action of the Sulfonylureas."

The GEORGIA DIABETES ASSOCIATION held a Scientific Program for the Georgia Academy of General Practice October 16 in Savannah. Following a welcome by John K. Davidson, M.D., the morning program presided over by Harold C. Atkinson, M.D., included: "What Is Diabetes Mellitus?" by Nathan M. DeVaughn, M.D.; "Diet Therapy in Diabetes Mellitus," by Thomas D. Johnson, M.D.; "Present Status of Oral Hypoglycemic Agents," by L. Harvey Hamff, M.D.; "Diabetes in Childhood," by Martin H. Smith, M.D.; "Pregnancy in the Diabetic Woman," by Jackson W. Landham, Jr., M.D.; "Diabetic Neuropathy," by Harry H. Brill, Jr., M.D.; and "Diabetic Acidosis," by Charles Warren Davidson, M.D.

The afternoon program, with Christopher J. McLoughlin, M.D., presiding, included: "Through the Years with Insulin," by Alex T. Murphy, M.D.; "Types of Insulin Available for Clinical Use," by Harold A. Ferris, M.D.; "Mode of Action of Insulin," by Rachmiel Levine, M.D., Chicago; "Insulin Hypoglycemia, Resistance, and Allergy," by Jules Victor, Jr., M.D.; and a panel discussion on "Questions and Answers about Insulin and Its Use Clinically," moderated by Walter L. Bloom, M.D., with Drs. Levine, Murphy, Ferris and Victor as participants. Dr. Levine presented a paper the previous day entitled "Fat Metabolism in Diabetes," at the Eleventh Annual Session of the Georgia Academy of General Practice which was held in Savannah October 14-15.

The NEW JERSEY DIABETES ASSOCIATION, in cooperation with the New Jersey State Department of Health, held The Seventh Annual Symposium on Lipids and Diabetes on Wednesday afternoon, October 21, in Newark. The program, under the chairmanship of Alfred E. Gras, M.D., included: "Fat: Its Relationship to the Treatment of Diabetes," by Leo P. Krall, M.D., Boston; "Some Aspects of Lipid Metabolism in Relation to Atherosclerosis," by Herbert J. Kayden, M.D., New York; "Effects of Fatty Infiltration of the Liver upon Sensitivity to Insulin Action," by Michael Somogyi, Ph.D., St. Louis; "Fat: The Liver and Diabetes," by Carroll M. Leevy, M.D.; and a panel discussion on "Lipids and Diabetes," moderated by Garfield G. Duncan, M.D., Philadelphia, with Drs. Krall, Kayden, Somogyi and Leevy as participants.

NEWS NOTES

PAN AMERICAN MEDICAL ASSOCIATION APPOINTS ADA MEMBERS

Bernardo A. Houssay, M.D., Ph.D., Buenos Aires, who received the 1947 Nobel Prize in physiology and medicine, heads the list of members of the Council of the Section on Endocrinology of the next Congress of the Pan American Medical Association in Mexico City, May 2-11, 1960. Other members of the Council are Charles H. Best, M.D., Toronto; Jerome W. Conn, M.D., Ann Arbor; Frank L. Engel, M.D., Durham, N.C.; S. O. Waife, M.D., Indianapolis; Manuel M. Villaverde, M.D., Havana; Alberto Viau, M.D., Guatemala City; and Laurance W. Kinsell, M.D., Oakland. Peter H. Forsham, M.D., San Francisco, has been appointed an officer of the Section.

Dr. Houssay and Frank Scott Perkin, M.D., Detroit, were recently elected to the Board of Trustees. Dr. Houssay is Director of the Instituto de Biologia y Medicina Experimental in Buenos Aires, and is on the Council of the Section on "Pharmacology and New Drugs," "Endocrinology," "Nutrition," and "Geriatrics and Gerontology" for the 1960 PAMA Congress.

PERSONALS

GARFIELD G. DUNCAN, M.D., Philadelphia, has been named head of the advisory editorial board which will direct compilation of material for the history of the Army medical department's role in the advancement of internal medicine during World War II.

RANDALL G. SPRAGUE, M.D., Rochester, Minnesota, was presented a Centennial Merit Award by Northwestern University Medical School on the occasion of the celebration of its Centennial on September 29, "in recognition of his substantial contributions to clinical medicine through research in metabolic and endocrine diseases and for his achievements as Professor of Medicine, Mayo Foundation, University of Minnesota."

GEORGE W. THORN, M.D., Boston, was presented the 1959 American Medical Writers' Association Honor Award "for distinguished contributions to medical literature" at the A.M.W.A. 16th Annual Meeting held in St. Louis October 2. Dr. Thorn was Banting Memorial Lecturer and the recipient of the Banting Medal at the Nineteenth Annual Meeting of the American Diabetes Association in Atlantic City June 6-7.

At the October meeting of the A.M.W.A., RICHARD H. ORR, M.D., New York, spoke on "The CBE Style Manual for Biologic Journals: A Progress Report" and on "Trends in Documentation: Their Effect on Medical Writing."

EDWARD TOLSTOI, M.D., is Chairman of the Committee on Laity Lectures for the Twenty-fifth Series of Lectures to the Laity of The New York Academy of Medicine. The series, entitled "On the Medical Frontiers," began on November 4 with The Linsly R. Williams Memorial Lecture entitled "Recent Advances in the Treatment and Prevention of Heart Diseases," by Paul D. White, M.D. Dr. Tolstoi was presiding chairman for this lecture. Among speakers for the series will be HERBERT POLLACK, M.D., who will speak on "Prevention and Treatment of Obesity," when he delivers The George R. Siedenburg Memorial Lecture on February 3.

NECROLOGY

CHENEY E. JOSEPH, Baton Rouge, Louisiana, born August 8, 1914.

SAYLOR J. MCGHEE, Lock Haven, Pennsylvania, born September 19, 1872.